Incident urogenital and anorectal Chlamydia trachomatis in women: the role of sexual exposure and autoinoculation: a multicentre observational study (FemCure)

Incident urogenital and anorectal *Chlamydia trachomatis* in women: the role of sexual exposure and autoinoculation: a multicentre observational study (FemCure)

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/sextrans-2021-055032).

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This study has in part been presented (plenary; oral) at the STI & HIV 2021 World Congress (ISSTDR), July 2021, AmsterdamAbstract published. Dukers-Muijrers NPL08. Chlamydia control in women and men who have sex with men: controversies and evidence on (extra)genital testing and treatment. Sexually Transmitted Infections 2021;97:A2. Supplement http://dx.doi.org/10.1136/sextrans-2021-sti.8

Received 21 April 2021
Accepted 8 November 2021
Published Online First 17 January 2022

To cite: Dukers-Muijrers NHTM, Schim van der Loeff M, Wolffs P, *et al*. *Sex Transm Infect* 2022;**98**:427–437.

ABSTRACT

Background Anorectal infections with *Chlamydia trachomatis* (CT) are common in women visiting STI outpatient clinics. We here evaluated the risk posed by sexual exposure and by alternate anatomical site infection for incident anorectal and urogenital CT.

Methods Prospective multicentre cohort study, FemCure. Participants were treated for CT, and after 4, 6, 8, 10 and 12 weeks, they self-collected anorectal and urogenital samples (swabs) for CT-DNA testing. We calculated the proportion with incident CT, that is, CT incidence (at weeks 6–12) by 2-week time-periods. Compared with no exposure (A), we estimated the risk of incident CT for (B) sexual exposure, (C) alternate site anatomic site infection and (D) both, adjusted for confounders and expressed as adjusted ORs with 95% CIs.

Results We analysed data of 385 participants contributing 1540 2-week periods. The anorectal CT incidence was 2.9% (39/1343) (95 CI 1.8 to 3.6); 1.3% (A), 1.3% (B), 27.8% (C) and 36.7% (D). The ORs were: 0.91 (95% CI 0.32 to 2.60) (B), 26.0 (95% CI 7.16 to 94.34) (C), 44.26 (95% CI 14.38 to 136.21) (D). The urogenital CT incidence was 3.3% (47/1428) (95% CI 2.4 to 4.4); 0.7% (A), 1.9% (B), 13.9% (C) and 25.4% (D). The ORs were: 2.73 (95% CI 0.87 to 8.61) (B), 21.77 (95% CI 6.70 to 70 71) (C) and 49.66 (95% CI 15.37 to 160.41) (D).

Conclusions After initial treatment, an alternate anatomical site CT infection increased the risk for an incident CT in women, especially when also sex was reported. This may suggest a key role for autoinoculation in the re-establishment or persistence of urogenital and anorectal chlamydia infections.

BACKGROUND

Chlamydia trachomatis (CT) infections continue to pose a significant public health problem.[1] There is an ongoing debate on the importance of anorectal CT in women, regarding its capacity to cause clinical disease and onward CT transmission.[2 3] Anorectal CT infections are common, with 8% positivity in women overall,[4] and 68% in women who have urogenital CT.[5] or with doxycycline 100 mg twice daily for 7 days is recommended as first-line treatment regardless anatomic site in UK and US, and for anorectal CT in Europe and Australia,[6–9] while the alternative is a single dose of azithromycin 1000 mg. Both treatments are highly effective in urogenital CT.[10] Two randomised controlled trials in Men who have sex with men (MSM) demonstrate substantial lower effectiveness of azithromycin in anorectal CT.[11 12] An observational study in women (FemCure spin-off) showed similar findings[13] and moreover demonstrated that CT treatment failures frequently (in 75%) represented viable CT.[4] In women, two-thirds of anorectal CT are missed in clinical practice as anorectal testing is not routinely recommended.[14–16] Most untested anorectal CT are in women who also have urogenital CT and who are (usually azithromycin) treated for their urogenital CT.[1 2] Anorectal CT may indirectly contribute to adverse reproductive outcomes if anorectal CT, by autoinoculation, leads to reinfection of the vagina.[17–19] Data are lacking that provide insight into the role of anorectal CT in urogenital CT (re-) infection risk and vice versa. We studied this in the FemCure study, following women for 12 weeks after initial CT treatment.[20]

METHODS

Study design

A prospective multicentre cohort study, FemCure.[20 21] We analysed data collected between week 4 and 12 after treatment (figure 1).

Regular STI clinic care

This study was conducted at three Centres for Sexual Health (STI clinics) in the Netherlands.[20] At the routine diagnostic clinic visit, women were tested for urogenital CT, and by clinic protocol, some women (based on report of unprotected anal sex with a (casual) sex partner, in case of sex work or when having rectal symptoms) were also tested for anorectal CT.[8 20]

Study enrolment

Women were enrolled between April 2016 and December 2017 at the treatment visit.[20] Eligible were non-pregnant adult women, 18 years or older and who had a urogenital or anorectal CT diagnosis.[21] Participation started after written informed consent.

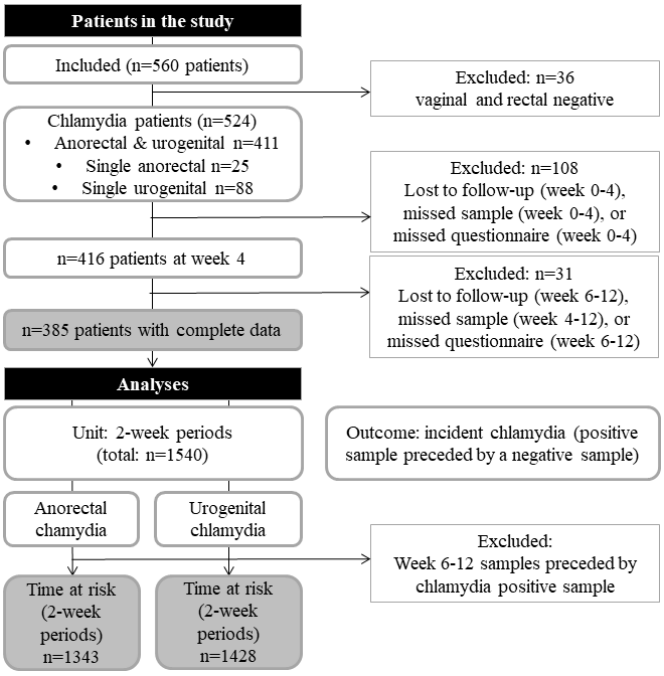

Figure 1 Flow chart of the number of patients in the study and 2-week periods in analyses.

Measurements

Women self-collected anorectal and urogenital swabs at enrolment at the clinic (week 0), immediately prior to treatment, and thereafter at weeks 4, 8 and 12 at the clinic, and at weeks 1, 2, 6 and 10 at home (figure 2). A nurse collected oropharyngeal swabs at clinic visits. Clinical and demographic data were collected at enrolment. Around each sample collection moment, patients completed a structured online questionnaire regarding their sexual practices in the preceding 2 weeks.[20] Study samples were tested batchwise afterwards; results were neither provided to clinicians nor to participants. At week 12, all participants received standard STI testing and, if indicated, treatment.

Laboratory analyses

Swabs were placed in COBAS buffer for testing with quantitative PCR (qPCR) testing detecting total CT-DNA (Roche Cobas 4800, Roche Diagnostics, Basel, Switzerland), according to manufacturers' guidelines. The qPCR cycle quantification (Cq) threshold values were taken as a proxy for bacterial load. Lower Cq values represents higher loads and vice versa.[22] At clinic visits, an additional self-taken swab (taken first) was immediately frozen at −80°C and later tested, using V-PCR, to detect 'viable CT', that is, CT-DNA from intact CT organisms.[23 24]

Ct sequence typing and semen biomarker testing

We performed multilocus sequence typing (MLST) on incident samples and the previous/current alternate anatomical site sample in the same woman; we only typed samples with a low Cq value (<32) because of many test fails with higher Cq values.[12 25 26] Samples of women with incident CT without reported sexual exposure risk were tested for y-chromosomal DNA with real-time PCR directed at amplification of a region of the Y chromosome targeting SRY at the sex determining region Y.[27] This semen biomarker can be detected for up to 14 days after condomless intercourse with a male partner.[27]

Outcome

The primary outcome was incident anorectal or urogenital CT. This was defined as a CT positive sample at week 6, 8, 10 or 12, which was preceded by a CT negative sample from the same anatomical site, that is, at week 4, 6, 8 or 10.

Main determinant

The main exposure determinant was constructed with four mutually exclusive categories: (A) no exposure, (B) sexual exposure, (C) exposure by alternate anatomical site infection and (D) both (online supplemental table 1).

Sexual exposure at the anorectal site was defined by sexual practices in past 2 weeks (collected at week 6–12) that we considered as potentially transmitting CT from a male partner to the anorectal site of the woman. This included:

1. Receptive anal intercourse or the woman being rimmed.
2. Vaginal intercourse or receiving cunnilingus as these sex practices occur close to the anorectal site.
3. Anogenital use of fingers or toys.

We included these sex practices irrespective of condom use, as condom use may not fully protect against CT transmission in case of condom slippage/breaking or when the penis touches anatomical sites of a woman during sex.

Sexual exposure at the urogenital site was defined by the same practices as mentioned above for the anorectal site.

Exposure by the alternate anatomical site infection for incident anorectal CT was defined as a urogenital CT at the current or previous 2 week visit, and for incident urogenital CT as a anorectal CT at the current or previous 2 week visit.

Secondary determinants

We varied the main determinant by restricting sexual exposure to condomless intercourse at the specific anatomical site. We also adapted the definitions of exposure by the alternate anatomical site infection by: (A) requiring presence of CT at the preceding visit and (B) stratifying the alternate anatomical site CT by Cq values (dichotomised by the median value

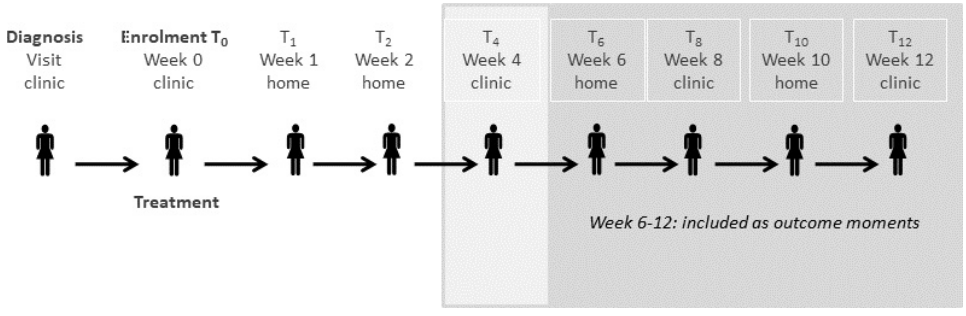

Figure 2 Study design of the FemCure study and at-risk 2-week periods for incident CT included in the analyses. T_n=time point at week n.

Table 1 Incidence of anorectal *Chlamydia trachomatis* (CT) by exposure category and cofactors for incident CT

	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence, %	OR (95% CI)	aOR (95% CI)
All periods at risk in analyses	39	1343	2.9		
Main determinant					
Exposure group				**p<0.001**	**p<0.001**
(A) No sexual exposure and no urogenital CT*	7	559	1.3	**1**	**1**
(B) Sexual exposure only*	9	717	1.3	1.00 (0.35 to 2.89)	0.91 (0.32 to 2.60)
(C) Urogenital CT only	5	18	27.8	**30.33 (7.72 to 119.10)**	**26.00 (7.16 to 94.34)**
(D) Sexual exposure and urogenital CT*	18	49	36.7	**45.79 (16.42 to 127.71)**	**44.26 (14.38 to 136.21)**
Secondary determinants					
Main determinant with diverse exposure definitions					
Exposure group (sex exposure=condomless anal intercourse)					
No condomless anal intercourse and no urogenital CT	16	1245	1.3	Na	
Condomless anal intercourse only	0	31	0		
Urogenital CT only	22	66	33.3		
Condomless anal intercourse and urogenital CT	1	1	100		
Exposure group: urogenital CT stratified by Cq value (load)					
No sexual exposure and no urogenital CT	7	559	1.3	Na	
Sexual exposure only	9	717	1.3		
Urogenital CT Cq-value >32 only†	2	15	13.3		
Urogenital CT Cq-value ≤32 only†	3	3	100.0		
Sexual exposure and urogenital CT Cq-value >32†	7	37	18.9		
Sexual exposure and urogenital CT Cq-value ≤32†	11	12	91.7		
Exposure group: urogenital CT stratified by viability					
No sexual exposure and no urogenital CT	7	559	1.3	Na	
Sexual exposure only	9	717	1.3		
Urogenital CT non-viable only‡	2	12	16.7		
Urogenital CT viable only	3	6	50.0		
Sexual exposure and urogenital CT non-viable‡	11	39	28.2		
Sexual exposure and urogenital CT viable	7	10	70.0		
Exposure group (urogenital CT at least at T_{n-2})				**p<0.001**	**p<0.001**
No sexual exposure and no previous urogenital CT	10	571	1.8	**1**	**1**
Sexual exposure only	17	734	2.3	1.33 (0.58 to 3.04)	1.20 (0.52 to 2.77)
Previous urogenital CT only	2	11	18.2	**12.47 (2.27 to 68.41)**	**10.73 (2.36 to 48.74)**
Sexual exposure and previous urogenital CT	10	27	37.3	**33.00 (12.44 to 87.52)**	**29.50 (9.97 to 87.22)**
Recent urogenital CT (indicative of autoinoculation)					
Urogenital CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	16	1276	1.3	**1**	**1**
Yes	23	67	34.3	**41.17 (18.95 to 89.43)**	**40.50 (18.24 to 89.88)**
Urogenital CT (at least at T_{n-2})				**p<0.001**	**p<0.001**
No	27	1305	2.1	**1**	**1**
Yes	12	38	31.6	**21.85 (10.06 to 47.50)**	**20.17 (9.00 to 45.24)**
Urogenital CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	16	1276	1.3	**1**	**1**
Yes, Cq-value >32†	9	52	17.3	**16.48 (6.74 to 40.32)**	**16.44 (6.02 to 44.90)**
Yes, Cq-value ≤32†	14	15	93.3	**1102.50 (151.08 to 8045.27)**	**1663.49 (274.50 to 10080.78)**
Urogenital CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	16	1276	1.3	**1**	**1**
Yes, non-viable‡	13	51	25.5	**26.94 (11.42 to 63.56)**	**25.94 (10.31 to 65.29)**
Yes, viable	10	16	62.5	**131.25 (31.86 to 540.71)**	**149.64 (33.03 to 677.98)**
Urogenital CT (at T_n or T_{n-2})					

Continued

Table 1 Continued

	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence, %	OR (95% CI)	aOR (95% CI)
No	16	1276	1.3	**1**	**1**
Yes, Cq-value >32 and non-viable†‡	5	6	83.3	**393.75 (42.52 to 3646.68)**	**467.00 (55.92 to 3913.83)**
Yes, Cq-value ≤32 or viable†	18	61	29.5	**32.97 (14.93 to 72.79)**	**31.88 (13.94 to 72.92)**
CT at week 0 and week 4: urogenital, anorectal or oral CT					
Urogenital CT at week 0 (enrolment) and 4 (treatment failure)				**p<0.001**	**p<0.001**
No	0	83	0	–	–
Yes, at week 0 but not at week 4	31	1207	2.6	**1**	**1**
Yes, at week 0 and at week 4 (treatment failure)	8	53	15.1	**6.74 (2.93 to 15.50)**	**6.44 (2.34 to 17.74)**
Anorectal CT at week 0 (enrolment) and 4 (treatment failure)				**p<0.001**	**p<0.001**
No	4	270	1.5	0.53 (0.18 to 1.53)	0.47 (0.16 to 1.35)
Yes, at week 0 but not at week 4	29	1041	2.8	**1**	**1**
Yes, at week 0 and at week 4 (treatment failure)	6	32	15.4	**8.05 (2.45 to 26.48)**	**8.45 (2.70 to 26.40)**
Oral CT at week 0 (enrolment)				p=0.760	
No	36	1221	2.9	1	
Yes	3	122	2.5	0.83 (0.25 to 2.75)	
Sex practices (past 2 weeks) (indicative of sexual route)					
Total number of sex partners				p=0.481	
0	12	578	2.1	1	
1	23	672	3.4	1.67 (0.82 to 3.43)	
2	3	70	4.3	2.11 (0.55 to 8.06)	
3–5	1	23	4.3	2.14 (0.29 to 15.61)	
New sex partners				p=0.176	
No new sex partners	29	1087	2.7	1	
1 new sex partner	7	219	3.2	1.21 (0.51 to 2.85)	
2–5 new sex partners	3	37	8.1	3.22 (0.94 to 11.05)	
Anal intercourse				p=0.952	
No	38	1293	2.9	1	
Yes, protected by condoms	1	32	3.1	1.07 (0.14 to 8.29)	
Yes, unprotected by condoms (condomless)	0	18	0	–	
Total number of times anal intercourse (anal sex acts)				p=0.832	
0	38	1293	2.9	1§	
1	1	42	2.4	0.81 (0.11 to 6.01)	
2–4	0	8	0	–	
Anogenital use toys/fingers				p=0.838	
No	35	1218	2.9	1	
Yes	4	125	3.2	1.12 (0.39 to 3.24)	
Being rimmed (oro-anal sex)				p=0.151	
No	36	1297	2.8	1	
Yes	3	46	6.5	2.44 (0.72 to 8.27)	
Vaginal intercourse				p=0.324	
No	13	584	2.2	1	
Yes, protected by condoms	21	565	3.7	1.16 (0.40 to 3.37)	
Yes, unprotected by condoms (condomless)	5	194	2.6	1.70 (0.83 to 3.46)	
Vaginal intercourse				**p=0.010**	**p=0.018**
No vaginal intercourse or protected with treated partner	14	606	2.3	**1**	**1**
Protected with untreated partner or condomless with treated partner	7	404	1.7	0.75 (0.29 to 1.91)	0.69 (0.27 to 1.80)
Condomless with untreated (or unknown treated) partner	18	333	5.4	**2.42 (1.18 to 4.94)**	**2.21 (1.07 to 4.58)**
Total times vaginal intercourse (vaginal sex acts)				p=0.426	
0	13	584	2.2	1	

Continued

Table 1 Continued

	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence, %	OR (95% CI)	aOR (95% CI)
1–2	8	204	3.9	1.79 (0.75 to 4.27)	
3–5	4	191	2.1	0.94 (0.32 to 2.80)	
6–9	5	172	2.9	1.32 (0.45 to 3.85)	
>=10	9	192	4.7	2.16 (0.88 to 5.31)	
Practice fellatio (oro-penile sex)				p=0.347	
No	21	825	2.5	1	
Yes	18	518	3.5	1.38 (0.71 to 2.69)	
Receiving cunningulus (oro-vaginal sex)				p=0.403	
No	24	912	2.6	1	
Yes	15	431	3.5	1.33 (0.68 to 2.62)	
Current clinical factors					
Urogenital symptoms				**p=0.035**	p=0.118
No	27	1103	2.4	**1**	1
Yes	12	240	5.0	**2.10 (1.05 to 4.18)**	1.82 (0.86 to 3.84)
Anorectal symptoms					
No	39	1338	2.9	Na	
Yes	0	5	0		
Currently having menses				p=0.219	
No	36	1151	3.1	1	
Yes	3	192	1.6	0.49 (0.16 to 1.52)	

Results of univariable and multivariable logistic regression analyses (with Odds Ratio (OR) and 95% CI).
*Sexual exposure (anal exposure): vaginal intercourse with or without condoms, anal intercourse with or without condoms, being rimmed, anogenital fingers/toys and receiving cunningulus.
†Cq value was averaged over T_n and T_{n-2} in case both time-points were CT positive.
‡Non-viable category also includes samples for which viability assessment could not be performed (this was only performed on subset of time-points).
§Not corrected for repeated measures due to problems with model convergence.
¶in Bold: statistically significant (p<0.05)
aOR, OR from the multivariable model that includes either the main determinant or any of the secondary determinants, and all potential confounders (treatment at week 0, study site, age, background and education); Cq, cycle quantification; Na, not applicable/not assessed.

of 32) and by viability test results. Other secondary determinants included a range of laboratory results, sexual practices and clinical factors, outlined in tables 1 and 2.[20]

Statistical analyses

The aim was to assess the risk of incident CT detection for categories A–D in the main determinant.

Data were analysed on the level of 2 week periods, and we used risk periods as unit of analysis. This means that for each sample taken at week 6, 8, 10 or 12, the sexual behaviour collected at the same time points (concerning the preceding 2-week interval), and CT at the alternate anatomical site (assessed at the same time or at the visit 2 weeks previously), were analysed (online supplemental table 1). We only analysed time at risk; for 2 weeks after a CT infection, people were not considered to be at risk.

We calculated proportions of incident CT by dividing the number of incident detections by the number of time at-risk periods, separately for each anatomical site.

To assess risks, we used multivariable logistic regression analyses controlling for repeated measurements in a person, using generalised estimating equations, expressing the odds of infection for each exposure category, relative to the reference category as ORs and 95% CI.

The main determinant and secondary determinants were evaluated.

The multivariable models included the main determinant and all potential confounders, that is, treatment type at week 0, study site, age, background and education.

We defined determinants as statistically significant, when the determinants' overall p was <0.05, or p was <0.10 when the determinant had more than two categories and p was <0.05 for at least one of the categories.

For patients with incident CT, we described and compared (using KrusKall-Wallis test) their median Cq values for various exposure categories. We also described the proportion of incident CT followed by positive samples and order of incident CT by anatomical site.

All analyses were performed using SPSS (IBM Corp. IBM SPSS Statistics for Windows, Version 24. Armonk, NY, USA).

RESULTS

Main population

In total, 1763 women were invited to participate, 560 (31.8%) were enrolled, and 416 women still participated at week 4.[12] Of those, 385 completed the study through samples and through questionnaires collected at weeks 6, 8, 10 and 12 (online supplemental table 2 for characteristics). They contributed 1343 at-risk periods to evaluate incident anorectal CT and 1428 risk periods to evaluate urogenital CT (figure 1).

Women with incident CT

There were 19 women who had incident anorectal CT only, of whom four had two incident CT, thus who in total had 23 incident anorectal CT (online supplemental figure 1). There were 29 women who had incident urogenital CT only, of whom four had two incident CT, thus who in total contributed 31 incident urogenital CT. There were 16 women who had both incident anorectal and

Table 2 Incidence of urogenital *Chlamydia trachomatis* (CT) by exposure category and cofactors for incident CT)

Total	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence %	OR (95% CI)	aOR (95% CI)
All periods at risk in analyses	47	1428	3.3		
Main determinant					
(A) No sexual exposure and no anorectal CT*	4	556	0.7	**1**	**1**
(B) Sexual exposure only*	14	722	1.9	2.73 (0.89 to 8.36)	2.73 (0.87 to 8.61)
(C) Anorectal CT only†	11	79	13.9	**22.32 (6.79 to 73.44)**	**21.77 (6.70 to 70.71)**
(D) Sexual exposure and anorectal CT~	18	71	25.4	**46.87 (14.69 to 149.49)**	**49.66 (15.37 to 160.41)**
Secondary determinants					
Main determinant with adapted exposure definitions					
Exposure group (sex exposure=condomless vaginal intercourse)				**p<0.001**	**p<0.001**
No condomless vaginal intercourse and no anorectal CT	8	744	1.1	**1**	**1**
Condomless vaginal intercourse only	10	534	1.9	1.76 (0.69 to 4.48)	1.72 (0.65 to 4.51)
Anorectal CT only	13	95	13.7	**14.59 (5.94 to 38.03)**	**14.15 (5.06 to 39.55)**
Condomless vaginal intercourse and anorectal CT	16	55	29.1	**37.00 (14.25 to 99.97)**	**38.61 (13.84 to 107.68)**
Exposure group: anorectal CT stratified by Cq value (load)					
No sexual exposure and no anorectal CT	4	556	0.7	Na	
Sexual exposure only	14	722	1.9		
Anorectal CT Cq-value >32 only	6	27	22.2		
Anorectal CT Cq-value ≤32 only	5	52	9.6		
Sexual exposure and anorectal CT Cq-value >32	7	23	30.4		
Sexual exposure and anorectal CT Cq-value ≤32	11	48	22.9		
Exposure group: anorectal CT stratified by viability					
No sexual exposure and no anorectal CT	4	556	0.7	Na	
Sexual exposure only	14	722	1.9		
Anorectal CT non-viable only‡	4	34	11.8		
Anorectal CT viable only	7	45	15.6		
Sexual exposure and anorectal CT non-viable‡	11	43	25.6		
Sexual exposure and anorectal CT viable	7	28	25.0		
Exposure group (anorectal CT at least at T_{n-2})				**p<0.001**	**p<0.001**
No sexual exposure and no previous anorectal CT	8	571	1.8	**1**	**1**
Sexual exposure only	21	734	2.3	2.07 (0.91 to 4.73)	2.06 (0.87 to 4.89)
Previous anorectal CT only	8	69	18.2	**9.23 (3.30 to 25.79)**	**7.72 (2.98 to 20.04)**
Sexual exposure and previous anorectal CT	10	54	37.3	**15.99 (5.99 to 46.05)**	**14.25 (5.03 to 40.35)**
Recent anorectal CT (indicative of autoinoculation)					
Anorectal CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	18	1278	1.4	**1**	**1**
Yes	29	150	19.3	**41.17 (18.95 to 89.43)**	**40.50 (18.24 to 89.88)**
Anorectal CT (at least at T_{n-2})				**p<0.001**	**p<0.001**
No	29	1305	2.2	**1**	**1**
Yes	18	123	14.6	**21.85 (10.06 to 47.50)**	**20.17 (9.00 to 45.24)**
Anorectal CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	18	1278	1.4	**1**	**1**
Yes, Cq-value >32	13	50	26.0	**24.60 (10.34 to 58.48)**	**26.10 (9.94 to 68.58)**
Yes, Cq-value ≤32	16	100	16.0	**13.33 (6.27 to 28.36)**	**14.18 (5.81 to 34.65)**
Anorectal CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	18	1278	1.4	**1**	**1**
Yes, non-viable‡	15	77	19.5	**16.94 (7.76 to 36.94)**	**17.85 (7.32 to 43.54)**
Yes, viable	14	73	19.2	**16.61 (7.20 to 38.31)**	**16.81 (6.59 to 42.90)**
Anorectal CT (at T_n or T_{n-2})				**p<0.001**	**p<0.001**
No	18	1278	1.4	**1**	**1**
Yes, Cq-value >32 and non-viable‡	13	66	19.7	**17.17 (7.56 to 39.01)**	**18.51 (7.27 to 47.15)**
Yes, Cq-value ≤32 or viable	16	84	19.0	**16.47 (7.50 to 36.17)**	**16.33 (6.61 to 40.34)**
CT at week 0 and week 4: urogenital, anorectal or oral CT					
Urogenital CT at week 0 (enrolment) and 4 (treatment failure)				p=0.716	
No	0	84	0	–	
Yes, at week 0 but not at week 4	46	1303	3.5	1	
Yes, at week 0 and at week 4 (treatment failure)	1	41	2.4	0.68 (0.09 to 5.33)	
Anorectal CT at week 0 (enrolment) and 4 (treatment failure)				**p<0.001**	**p<0.001**
No	4	266	1.5	0.53 (0.18 to 1.53)	0.47 (0.16 to 1.35)

Continued

Table 2 Continued

Total	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence %	OR (95% CI)	aOR (95% CI)
Yes, at week 0 but not at week 4	24	1030	2.3	**1**	**1**
Yes, at week 0 and at week 4 (treatment failure)	19	132	14.4	**8.05 (2.45 to 26.48)**	**8.45 (2.70 to 26.40)**
Oral CT at week 0 (enrolment)				p=0.398	
No	41	1296	3.2	1	
Yes	6	132	4.5	1.46 (0.61 to 3.49)	
Sex practices (past 2 weeks) (indicative of sexual transmission)					
Total number of sex partners				**p=0.027**	**p=0.013**
0	15	639	2.3	**1**	**1**
1	23	688	3.3	1.44 (0.72 to 2.86)	1.42 (0.70 to 2.87)
2	7	75	9.3	**4.28 (1.65 to 11.14)**	**4.81 (1.78 to 13.02)**
3–5	2	26	7.7	3.47 (0.73 to 16.54)	**5.18 (1.07 to 25.15)**
New sex partners				**p=0.019**	**p=0.011**
No new sex partners	32	1159	2.8	**1**	**1**
1 new sex partner	10	228	4.4	1.62 (0.78 to 3.33)	1.72 (0.84 to 3.55)
2–5 new sex partners	5	41	12.2	**4.89 (1.53 to 15.64)**	**5.50 (1.70 to 17.80)**
Anal intercourse					
No	47	1379	3.4	Na	
Yes, protected by condoms	0	18	0		
Yes, unprotected by condoms (condomless)	0	31	0		
Total number of times anal intercourse (anal sex acts)					
0	47	1379	3.4	Na	
1	0	40	0		
2–4	0	9	0		
Anogenital use toys/fingers				p=0.883	
No	43	1298	3.3	1	
Yes	4	130	3.1	0.92 (0.34 to 2.56)	
Being rimmed (oro-anal sex)					
No	47	1383	3.4	Na	
Yes	0	45	0		
Vaginal intercourse				p=*0.171*	
No	16	642	2.5	1	
Yes, protected by condoms	5	197	2.5	1.02 (0.36 to 2.89)	
Yes, unprotected by condoms (condomless)	26	589	4.4	1.81 (0.93 to 3.52)	
Vaginal intercourse				**p=0.056**	**p=0.081**
No vaginal intercourse or protected with treated partner	16	665	2.4	1	1
Protected with untreated partner or condomless with treated partner	12	411	2.9	1.22 (0.56 to 2.67)	1.30 (0.58 to 2.88)
Condomless with untreated (or unknown treated) partner	19	352	5.4	**2.31 (1.13 to 4.75)**	**2.32 (1.09 to 4.92)**
Total times vaginal intercourse (vaginal sex acts)				p=0.452	
0	16	642	2.5	1	
1–2	10	217	4.6	1.89 (0.83 to 3.31)	
3–5	9	203	4.4	1.82 (0.78 to 4.25)	
6–9	7	174	4.0	1.64 (0.65 to 4.14)	
≥10	5	192	2.6	1.05 (0.37 to 2.95)	
Practice fellatio (oro-penile sex)				p=0.074	
No	23	888	2.6	1	
Yes	24	540	4.4	1.75 (0.95 to 3.23)	
Receiving cunningulus (oro-vaginal sex)				**p=0.013**	**p=0.006**
No	24	981	2.4	**1**	**1**
Yes	23	447	5.1	**2.16 (1.78 to 3.97)**	**2.46 (1.29 to 4.69)**
Current clinical factors					
Urogenital symptoms				p=0.737	
No	38	1181	3.2	1	
Yes	9	247	3.6	1.14 (0.54 to 2.41)	
Anorectal symptoms					
No	43	1423	3.3	Na	
Yes	0	5	0		
Current having menses				p=0.127	

Continued

Table 2 Continued

Total	Incident CT, n	Time (2 weeks) periods at risk, n	Incidence %	OR (95% CI)	aOR (95% CI)
No	44	1233	3.6	1	
Yes	3	205	1.5	0.40 (0.12 to 1.30)	

Results of univariable and multivariable logistic regression analyses (with Odds Ratio (OR) and 95% CI).
*Sexual exposure (anal exposure): vaginal intercourse with or without condoms, anal intercourse with or without condoms, being rimmed, anogenital fingers/toys and receiving cunningulus.
†One incident CT showed a different strain than observed at the vagina at week 0, which may point to sexual exposure; thus, one case might have been misclassified from group D.
‡Non-viable category also includes samples for which viability assessment could not be performed (this was only performed on subset of time-points).
§Cq value was averaged over T_x and T_{x-2} in case both time-points were CT positive.
¶In Bold: statistically significant ($p<0.05$)
aOR, OR from the multivariable model that includes the main determinant or any of the secondary determinants, and all potential confounders (treatment at week 0, study site, age, background and education); Cq, cycle quantification; Na, not applicable/not assessed.

urogenital CT (none had more than one incident CT at an anatomical site). In total, 64 women contributed incident CT; 35 women contributed 39 incident anorectal CT and 45 women contributed 47 incident urogenital CT.

Incident anorectal CT and associated factors

The proportion of 2-week risk periods with incident anorectal CT was 2.9% (39/1343) (95% CI: 2.1 to 3.9). The odds for incident anorectal CT were higher with a previous/current urogenital CT (exposure category C) and when having both a previous/current urogenital CT and sexual exposure (D) (table 1).

Evaluating secondary determinants, adjusting for potential confounders, incidence of anorectal CT was higher in women who had a previous/current urogenital CT, especially with a low urogenital CT Cq value (proxy for higher CT load) or with viable urogenital CT present. Moreover, incident anorectal CT was associated with a longer period with the previous CT result, that is, anorectal or urogenital CT as measured at week 4. Finally, incident anorectal CT was associated with having condomless vaginal intercourse with an untreated (or unknown treatment status) sex partner.

Table 3 Number and proportion of incident *Chlamydia trachomatis* (CT) infections at week 6–12 after treatment, by initial treatment type (at week 0), by test result at enrolment (week 0) and result at week 4 (ie, likely treatment failure in case positive at week 4), in 385 women, FemCure, 2016–2017

	Incident anorectal CT at 6–12 weeks after treatment			Incident urogenital CT at 6–12 weeks after treatment		
	Incident CT	Time (2 weeks) periods at risk	Incidence	Incident CT	Time (2 weeks) periods at risk	Incidence
By week 0 and 4 result, and initial treatment (N= number of patients)	n	N	%	n	N	%
Azithromycin treated at week 0 (n=245)						
Patients with anorectal and urogenital CT at week 0 (n=192)	**24**	**601**	**4.0**	**34**	**696**	**4.9**
And anorectal CT positive at week 4 (n=40)	6	17	35.3	19	113	16.8
And anorectal negative at week 4 (n=152)	18	584	3.1	15	583	2.6
And urogenital CT positive at week 4 (n=14)	3	31	9.7	0	30	0
And urogenital CT negative at week 4 (n=178)	21	570	3.7	34	666	5.1
Patients with single anorectal CT at week 0 (n=1)	**0**	**4**	**0**	**0**	**4**	**0**
And anorectal CT positive at week 4 (n=0)	0	0	0	0	0	0
And anorectal CT negative at week 4 (n=1)	0	4	0	0	4	0
Patients with single urogenital CT at week 0 (n=52)	**4**	**202**	**2.0**	**2**	**198**	**0.5**
And urogenital CT positive at week 4 (n=2)	2	5	40.0	0	1	0
And urogenital CT negative at week 4 (n=50)	2	197	1.0	2	197	1.0
Doxycycline treated at week 0 (n=140)						
Patients with anorectal and urogenital CT at week 0 (n=102)	**11**	**389**	**2.8**	**9**	**382**	**2.4**
And anorectal CT positive at week 4 (n=4)	0	12	0	0	15	0
And anorectal negative at week 4 (n=98)	11	377	2.9	9	367	2.5
And urogenital CT positive at week 4 (n=5)	3	16	18.8	0	9	0
And urogenital CT negative at week 4 (n=97)	8	373	2.1	9	373	2.4
Patients with single anorectal CT at week 0 (n=20)	**0**	**79**	**0**	**0**	**80**	**0**
And anorectal CT positive at week 4 (n=1)	0	3	0	0	4	0
And anorectal CT negative at week 4 (n=19)	0	76	0	0	76	0
Patients with single urogenital CT at week 0 (n=18)	**0**	**68**	**0**	**2**	**68**	**2.9**
And urogenital CT positive at week 4 (n=1)	0	1	0	1	1	100.0
And urogenital CT negative at week 4 (n=17)	0	67	0	1	67	1.5
bold indicates the totals (numbers and proportions)						

Table 4 Number of incident anorectal and urogenital *Chlamydia trachomatis* (CT) infections, test result of subsequent samples and cycle quantification (CQ) threshold of CT positive samples, in women at 6–12 weeks after treatment

	Incident CT	Incident CT with subsequent sample(s)	Incident CT with subsequent sample negative	Incident CT with subsequent sample positive	Incident anorectal only	Incident anorectal first then urogenital	Incident urogenital first then anorectal	Incident both at the same visit	Incident urogenital only
Anorectal CT									
	N	N	n	n/N (%)	n/N	n/N	n/N	n/N	n/N
Total	39	33	10	23/33 (69.7)	23/39	1/39	4/39	11/39	0
By rectal exposure*									
A. No sexual exposure	7	5	2	3/9 (60.0)	7/7	0/7	0/7	0/7	
B. Sexual exposure only	9	8	5	3/4 (37.5)	8/9	1/9	0/9	0/9	
C. Urogenital CT only	5	4	1	3/8 (75.0)	2/5	0/5	0/5	3/5	
D. Sexual exposure and urogenital CT	18	16	2	14/12 (87.5)	6/18	0/18	4/18	6/18	
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)					
Cq value	37.9 (35.9–39.1)	37.9 (35.9–39.3)	38.7 (37.9–39.6)	37.2 (34.7–38.7)†					
Vaginal CT									
	N	N	n	n/N (%)		n/N	n/N	n/N	n/N
Total	47	40	18	22/40 (55.0)	0	1/47	4/47	11/47	31/47
By vaginal exposure									
A. No sexual exposure	4	3	2	1 (33.3)		0/4	1/4	0/4	3/4
B. Sexual exposure only	14	12	6	6 (50.0)		0/14	3/14	0/14	11/14
C. Anorectal CT only	11	9	6	3 (33.3)		0/11	0/11	3/11	8/11
D. Sexual exposure and anorectal CT	18	16	4	12 (75.0)		1/18	0/18	8/18	9/18
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)					
Cq value	36.2 (30.3–38.9)	36.7 (31.2–38.8)	38.7 (37.0–39.2)	31.6 (28.8–36.1)‡					

*Sexual exposure: vaginal intercourse, anal intercourse, receptive oro-anal sex, anogenital fingers/toys and cunningulus.
†P=0.042 compared with incident CT with subsequent sample negative; lower Cq value represents higher bacterial load.
‡P<0.001 compared with incident CT with subsequent sample negative; lower Cq value represents higher bacterial load.

Incident urogenital CT and associated factors

The proportion of 2-week risk periods with incident urogenital CT between week 6–12 post-treatment was 3.3% (47/1428) (95% CI 2.4 to 4.4).

The odds for having incident urogenital CT were higher with a previous/current anorectal CT (C) and when having both a previous/current anorectal CT and sexual exposure (D) (table 2).

Evaluating secondary determinants, adjusting for potential confounders, incidence of urogenital CT was higher with a previous/current anorectal CT and when having both a previous/current anorectal CT and condomless vaginal intercourse. Incidence was higher in women who had a previous/current anorectal CT regardless of anorectal CT Cq-value or viability. Moreover, incident urogenital CT was associated with a anorectal CT at week 4, representing possible anorectal treatment failure. Other sexual exposure factors found to be associated were two or more recent sex partners, having condomless vaginal intercourse with an untreated (or unknown treatment status) sex partner and receiving cunnilingus.

See table 3 for anorectal and urogenital CT incidence by moment of previous test result and initial treatment.

y-DNA assessment and sequence typing

All 12 incident anorectal CT samples and 15 incident urogenital CT samples that had no sexual exposure reported (groups A and C), as well as their same-week collected alternate anatomical site sample, were assessed for y-DNA. None contained y-DNA. We could evaluate by MLST: 6/23 patients with incident anorectal CT who also had urogenital CT, and 5/29 patients with incident urogenital CT who also had anorectal site CT both sites. In all, the strain was the same at the two anatomical sites. In 32 women with a typed incident CT, we could also type the week 0 samples; of these, eight had a different strain at follow-up (of whom seven reported recent sex) (see online supplemental table 3 and online supplemental figure 1).

Chlamydia and load in incident and subsequent positive samples

No women with incident anorectal CT reported anal symptoms. Nine (19.1%) women with incident urogenital CT reported urogenital symptoms. Of women with incident anorectal CT, 69.7%, and of women with incident urogenital CT, 55.0% (p=0.007) had subsequent positive samples (table 4). Incident samples with subsequent positive samples showed a lower median Cq value compared with incident samples with subsequent negative samples.

DISCUSSION

This prospective observational multicentre study in female outpatient STI clinic attendees assessed the incidence of anorectal and urogenital CT between 6 and 12 weeks after regular treatment and assessed the risk of sexual exposure, of an alternate site CT infection in a woman, and the combination of both.

For each 2-week period (at risk), anorectal CT incidence was 2.9% and urogenital CT incidence was 3.3%. The risk for incident CT was increased in women who did not report sex but had a previous/current CT at the alternate anatomical site. This may suggest autoinoculation, from the anorectal to the urogenital site and vice versa, in which factors as hygiene practices may also play a role. The risk for incident CT further increased when a woman had CT at the alternate site and also reported sex. This may reflect sexual transmission, autoinoculation, or both, for example, sex enhanced autoinoculation. Notably, a high vaginal CT load (or when viable) increased the risk for incident rectal CT even further. This study is the first study in human data to provide evidence for an autoinoculation process and in line with previous estimated

probabilities (1% per day) in a mathematical model.[28] We also evaluated various sexual practices, and several (unprotected sex with an untreated partner, cunnilingus and ≥2 recent sex partners) were associated with incident anorectal or vaginal CT. Unfortunately, we could not assess the contributions of the sexual practices independently of each other and independently of the alternate anatomical site CT.

Alternate anatomical site CT may have been caused by sexual transmission but also by failed initial treatment, as indicated by CT at week 4. Indeed, having an anorectal CT at week 4 (indicating treatment failure) was associated with an incident vaginal CT. Of incident urogenital CT who had anorectal CT exposure, 8/11 (group C) and 9/18 (group D) had anorectal CT at week 4. Of incident anorectal CT who had urogenital CT exposure, 2/5 (group C) and 6/18 (group D) had urogenital CT at week 4.

Strengths of this study include the study population, that is, women, for whom scarce anorectal CT data are available and the multicentre design involving three large STI clinics. Also, the rigorous and detailed prospective data collection, including two alternate anatomical sites within one study, exploring sexual transmission and autoinoculation simultaneously and adjustment for putative confounders are strengths. We studied a range of possible exposures, including sexual practices, and CT at various anatomical sites, CT load and viability.

We also recognise limitations. Confounding could not be ruled out but minimised by adjusting for various factors in analyses. Misclassification of exposures could have occurred. While underreporting of anal sex is possible, this should not lead to major bias since women who practice anal sex usually also practice vaginal sex,[29] and in the sexual exposure, we also included women who had vaginal sex. In incident CT without reported sexual exposure, no y-DNA was detected. Still, there were few incident CT without any exposure (group A), that is, 18%, 7/39 of anorectal incident CT and 9%, 4/47 of urogenital incident CT. These might represent a possible persistent infection with on–off effects in the qPCR around the detection limit. Another explanation is transmission by unreported sex where y-DNA could no longer be detected (eg, cunningulus at 14 days ago) or when the y-DNA was cleared within 14 days. Exposure misclassification could also have occurred by our definition of exposure by the alternate anatomical site infection, that is, previous/current alternate site CT. However, exclusion of the current alternate site CT still revealed strong associations. To further exclude potential bias, we performed sequence typing and, though incomplete, MLST revealed the same strains in both incident and alternate anatomic site CT in a woman; of note, a same strain does not rule out a possible reinfection from the same partner. Outcome misclassification may also be present. As our NAAT did not have an internal human cell detection control, we could not rule out that negative NAAT results were due to inadequate self-sampling. Possibly, we may have had false negative/positive results in samples with a low bacterial load around the detection limit. We acknowledge that, by using self-collection methods to assess CT, we theoretically might have missed infections higher in the columnal cells. Also, we acknowledge that NAAT detects CT-DNA, thus viable or non-viable CT. Finally, participants originated from STI clinic practice and likely are not fully representative for the general population.[20] Compared with all CT diagnosed STI clinic women, in FemCure, women with either high or low educational level (compare to middle), women without a history of STI and non-Western migrant women were under-represented.[21]

What are clinical implications? Call is to focus CT management on preventing sequelae.[1] Repeat urogenital infections may increase the risk for sequelae.[19 28] Persisting undetected anorectal CT may, in initially urogenital CT treated women, lead to subsequent reinfection of the urogenital site. Autoinoculation might be mechanically enhanced by sex, suboptimal hygiene practices or represent a ping-pong effect though an intermediate host, that is, a sexual partner. Moreover, it is possible that urogenital reinfections are acquired via a CT infected or transient positive sexual partner. Any CT management strategy in women should include partner treatment and advise condom use with new partners and untreated partners. In women themselves, we may need to extend CT management to treating untested anorectal CT, using a treatment that is optimal for both urogenital and anorectal CT. This would mean abandoning azithromycin and moving to universal doxycycline as now recommended by UK and US guidelines.[6 9] When such a universal treatment strategy is accompanied by selective anorectal testing to detect and treat (at least part of the) single anorectal CT, this would leave only a very small part of all anorectal CT untreated. The importance of single anorectal CT infections in women is not straightforward. Single anorectal CT is not common and may clear spontaneously (28%) in women.[30] Whether universal doxycycline use, potentially in combination with selective anorectal testing, in women is cost-effective is unknown. Furthermore, even though anorectal CT is prevalent, often untested, untreated or suboptimally treated, we acknowledge that the absolute numbers of anorectal CT that will lead to an incident and persisting urogenital CT and, most important, sequelae, is unknown. Finally, universal doxycycline use in women should be carefully evaluated, given some limitations, as described before.[12]

In conclusion, in the context of current routine STI clinic care, CT re-exposure by a treated woman's own alternate anatomical site, alone or in combination with sexual practices may play a key role in the persistence of chlamydia infection in women.

Key messages

- ⇒ In women, azithromycin treatment in anorectal chlamydia is unsuccessful in about 20%, with the potential of subsequent reinfection of the vagina through autoinoculation.
- ⇒ In women followed 2-weekly between week 4–12 after initial treatment, anorectal CT incidence was 2.9% (39/1343), and urogenital CT incidence was 3.3% (47/1428).
- ⇒ Incident anorectal and urogenital CT were associated with a previous *Chlamydia trachomatis* at the alternate anatomical site, suggesting autoinoculation, as well as with sexual exposure.
- ⇒ We found that 55% of incident urogenital and 70% of incident anorectal infections subsequently persisted for at least 2 weeks.
- ⇒ Autoinoculation of chlamydia from the rectum to the vagina and vice versa may play a key role in the re-establishment or persistence of chlamydia infection in women, in case of suboptimal treatment or lack of anorectal testing, or in case of sexual (re-)exposure.

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Acknowledgements We are grateful to the staff at the Public Health Service (GGD) South Limburg: Ronald van Hoorn, Maria Mergelsberg, Mandy Sanders, Emily Suijlen, Bianca Penders, Mieke Steenbakkers, Helen Sijstermans, Ine de Bock, Lisanne Eppings, Julien Wijers, Patricia Zaandam, Jeanine Leenen, Jeanne Heil, Stephanie Brinkhues and Genevieve van Liere; the staff at GGD Rotterdam-Rijnmond: Astrid Wielemaker, Angie Martina, Roselyne Uwimana, Mieke Illidge and Klaas de Ridder; and the staff at GGD Amsterdam, Arjan Hogewoning, Dieke Martini, Myra van Leeuwen, Claudia Owusu, Jacqueline Woutersen, Princella Felipa, Mayam Amezian, Arjdal Khadija, Luann Noordpool and Iris Deen, who were involved in the logistics, recruitment and enrolment of the study, and Martijn van Rooijen for data management, and Anders Boyd for statistical advice. We would also like to thank the staff at the laboratories of Medical Microbiology of the Maastricht University Medical Centre, especially Judith Veugen, Laura Saelmans and Kevin Janssen. Additionally, we would like to thank the staff of the microbiological laboratory of the GGD Amsterdam, especially Esther Heuser and Michelle Himschoot. We would like to thank Jos Herbergs of the laboratory DNA-Lysis for Y-DNA analyses. Finally, we would like to thank Jan van Bergen, Servaas Morre and Birgit van Benthem for their initial involvement in the study design.

Contributors NHTMD-M wrote the report and performed the statistical analyses. NHTMD-M and MSvdL designed the statistical analysis. PW, ML and SMB set up and performed the laboratory analyses. NHTMD-M, CJPAH, HMG and HDV supervised the study. All authors reviewed the results, provided guidance on the method, and drafted, reviewed and provided critical feedback on the report. NHTM-D is guarantor of the study

Funding This study is funded by a governmental organisation grant from the Netherlands: Organisation for Health Research and Development (ZonMW Netherlands, registration number 50-53000-98-109 and 52-2008-002).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval All participants provided written informed consent. This study was approved by the Medical Ethical Review Committee (IRB) from the Maastricht University Medical Centre (MUMC+), Maastricht, the Netherlands (NL51358.068.15/METC153020, 20-01-2016). Study oversight: this study was monitored by the Clinical Trial Centre Maastricht (Maastricht University).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. Coded data are available to interested researchers on reasonable request. Please send an email to helen.sijstermans@ggdzl.nl.

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