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Men and Women Repeatedly Infected With *Chlamydia trachomatis* Have a Lower Urogenital Bacterial Load

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Abstract: We assessed whether patients repeatedly infected with *Chlamydia trachomatis* (CT) have a lower urogenital or anorectal CT load. A CT-positive retest was independently associated with higher vaginal and higher urine Cq values ($P < 0.01$). Partial immunity could play a role in repeat urogenital infections, potentially not in anorectal infections.

Repeat *Chlamydia trachomatis* (CT) infections remain a challenge in CT control. Repeat CT infections can be caused by reinfections from an untreated sexual partner, new infection from the regular or new partner, or a persistent infection due to treatment failure. Repeat CT infections are common; up to 32% of patients with CT infection test positive again within 1 year of diagnosis.^{1,2} Repeat infections increase the risk of developing serious reproductive sequelae.³ Therefore, retesting CT-positive patients within 3 to 12 months after initial test is an effective control strategy and can be used to enhance the population-based prevention of CT.⁴ Although retesting is advised in international guidelines, retesting rates remain typically low (15%–40%).⁴

A high CT bacterial load (hereafter, CT load), frequently expressed as the number of CT bacteria/mL, could potentially affect transmission and occurrence of sequelae.⁵ Some studies suggest that repeat CT infections are associated with a lower CT load potentially due to partial immunity against CT.^{6,7} Nevertheless, these studies on CT load and repeat infections had some limitations like including only small numbers of patients, presenting only univariable analyses, or only assessing the urogenital site of women.^{6,7} Furthermore, data on repeat anorectal infections are lacking, although these infections are common among both men and women who have sex with men (MSM).⁸ Therefore, we

studied CT load in a relatively large number of both repeat positive urogenital and anorectal samples from men and women.

MATERIALS AND METHODS

Study Population

Data from 6170 CT-positive patients (aged ≥ 16 years) with 7224 CT-positive consultations from January 2012 to June 2018 were used to derive our study population of repeat positives in both men and women from urogenital and anorectal samples. These data originated from the laboratory registry of the Medical Microbiology Laboratory of Maastricht University Medical Center from a total of 62,306 CT tests with a CT positivity of 11.6%.⁹

All samples were tested for CT with the same nucleic acid amplification test (NAAT) for both plasmid and chromosomal DNA (COBAS 4800; Roche Diagnostics, Basel, Switzerland), as per the manufacturer's protocol.¹⁰ We used the NAAT-derived Cq value as a proxy for CT load.⁹

For women, CT-positive vaginal swabs and CT-positive anorectal swabs were included. For men, CT-positive first void urine samples and CT-positive anorectal swabs were included. The first positive CT test of a patient was defined as the "initial positive test." Only patients with at least one CT-positive retest were included in the study population. Samples that tested positive for CT within 30 days of a previous positive CT sample were excluded to rule out positivity because of possible false-positive results of nonviable recurrent CT ($n = 230$ vaginal swabs, $n = 76$ urine samples, and $n = 66$ anorectal swabs). All positive samples taken later than 30 days after the initial positive test were considered as a "positive retest" in the entire study period.

In total, $n = 429$ patients with at least one urogenital or anorectal CT-positive retest were included. Of this sample, 51.5% were men, the mean age was 28 years, and most had a Western ethnicity (77.2%). Furthermore, 88.1% were diagnosed at the sexually transmitted infection clinic, 9.3% by the general practitioner, and 2.6% by hospital medical specialists. Of the patients diagnosed at the sexually transmitted infection clinic, 26.4% were heterosexual men, 26.6% were MSM, and 47.0% were women.

Of the 137 men with at least 1 positive urogenital CT retest, 127 had 1 positive urine retest, and 10 had 2 positive retests (total: $n = 147$). Of the 84 men with at least 1 positive anorectal CT retest, 63 had 1 positive retest, 16 had 2 positive retests, and 5 had 3 positive retests (total: $n = 110$).

Of the 187 women with at least 1 positive vaginal CT retest, 170 had 1 positive retest, 16 women had 2 positive retests, and 1 woman had 3 positive retests (total: $n = 205$). Of the 21 women with at least 1 positive anorectal CT retest, 19 had 1 positive retest, 1 had 2 positive retests, and 1 woman had 3 positive retests (total: $n = 24$).

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Cycle Quantification Validation for CT Load

We compared the Cq values obtained from the diagnostic NAAT with previously determined quantitative in-house polymerase chain reaction, to ensure that the Cq value can indeed be used as a proxy for CT load in the different sample types.^{10–12} In brief, we quantified CT load by an in-house TaqMan real-time polymerase chain reaction to quantify CT *OmpA*-gene copies/mL.¹⁰ A full description of the CT load quantification has been described elsewhere.¹⁰ In an earlier publication, we described the correlations between Cq values and CT load per sample type in detail.⁹ Overall, high correlations were observed (Pearson $r = >0.88$), except for urine samples (Pearson $r = 0.61$).⁹

Statistical Analyses

The main determinant tested was positive retest (binary), in which the initial positive test was used as the reference group and any subsequent positive CT test as the group of interest per sample type. In the main analyses, the association between positive retest and the outcome measure “Cq values,” as a proxy for CT load, was assessed using generalized linear models for repeated measures per sample type (vaginal swab, urine sample, and anorectal swab). The analyses data set was aggregated on a consultation level. Every subsequent CT-positive consultation of the same individual, later than at least 30 days of a previous consultation, was counted as a positive retest when the same sample type as at the initial consultation was taken. We adjusted for sex, age (continuous), year of consultation (continuous), number of days between subsequent CT infection (continuous), concurrent anorectal CT, concurrent oropharyngeal CT, coinfection with urogenital NG, coinfection with anorectal NG, coinfection with oropharyngeal NG, and coinfection with HIV. Only multivariable results are presented. A P value of <0.05 was considered statistically significant. Mean

values, SDs, (adjusted) mean differences, and 95% confidence intervals (CI) were calculated.

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

Medical Ethical Clearance

The Medical Ethics Committee of the Maastricht University Medical Center (Maastricht, the Netherlands) approved this study (METC 2017-0251) and waived the need for individual written patient consent. Because the retrospective data originated from regular care and were analyzed anonymously, no further informed consent for data analysis was obtained.

RESULTS

Men

In multivariable analyses, a positive urine retest ($n = 147$) was associated with higher Cq values (mean difference, 1.08 Cq; 95% CI, 0.27–1.90; $P = 0.009$) compared with the initial positive urine test ($n = 137$; Fig. 1). In a sensitivity analyses, by including only the first positive urine retest, results were similar (mean difference, 1.00 Cq; 95% CI, 0.17–1.82; $P = 0.02$).

In multivariable analyses, a positive anorectal retest ($n = 110$) was not associated with Cq values (mean difference, 0.10 Cq; 95% CI, -1.15 to 1.35 ; $P = 0.88$; Fig. 1). In a sensitivity analyses, by including only the first positive anorectal retest, results were similar and not statistically significant.

Women

In multivariable analyses, a CT-positive vaginal retest ($n = 205$) was associated with higher vaginal Cq values compared with the initial positive vaginal test ($n = 187$; mean difference, 1.51

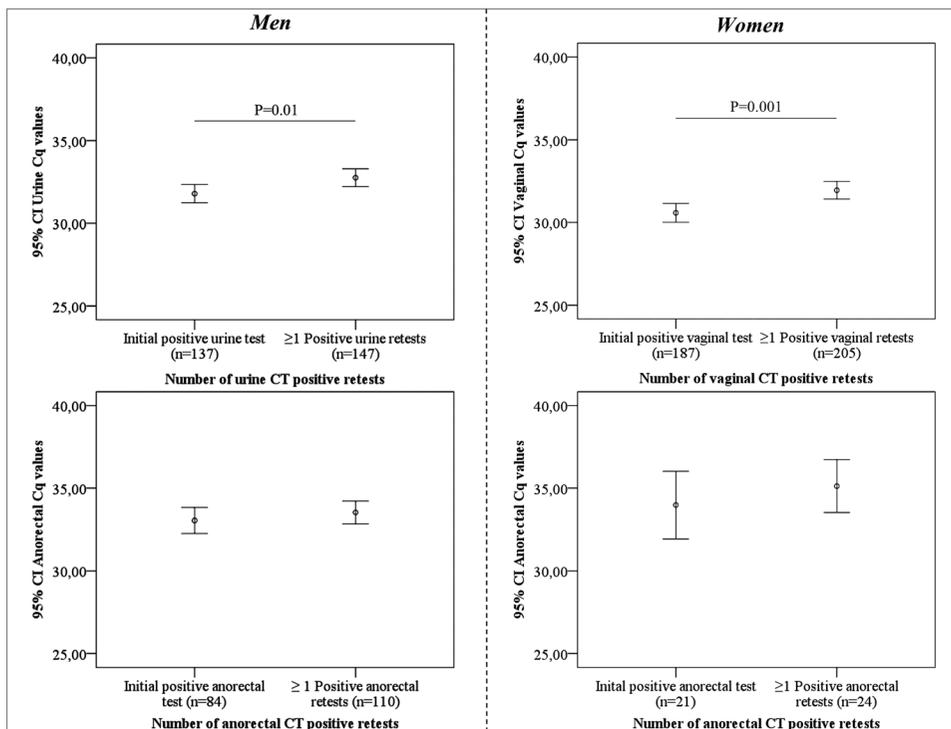


Figure 1. Errors bars with 95% CIs showing the CT bacterial load, estimated by Cq values, of the initial positive test and ≥ 1 repeat infections of the urogenital and anorectal site of men and women.

Cq; 95% CI, 0.58–2.44; $P = 0.002$) (Fig. 1). In a sensitivity analyses, by including only the first positive vaginal retest, results were similar (mean difference, 1.36 Cq; 95% CI, 0.42–2.30; $P = 0.004$).

In multivariable analyses, a CT-positive anorectal retest ($n = 24$) was not associated with Cq values in women (mean difference, 1.00 Cq; 95% CI, -1.87 to 3.87 ; $P = 0.49$; Fig. 1). In a sensitivity analyses, by including only the first positive anorectal retest, results were similar and not statistically significant.

DISCUSSION

This is the largest study to date assessing the CT bacterial load, estimated by Cq values, of repeat CT infections per sample type adjusted for potential confounders. Men and women repeatedly infected with CT have higher urogenital Cq values and likely a lower CT load. However, this was not true for anorectal samples, as no difference was observed in Cq values in anorectal samples of both men and women.

A strength of our study is the relatively large number of repeat infections analyzed stratified per sample type. Our multivariable analyses confirm the univariable findings of smaller studies indicating that women repeatedly infected with CT have a lower urogenital CT load.^{6,7} To our knowledge, this is the first study showing that also men repeatedly infected with CT have a lower urogenital CT load.

A limitation was that the Cq values of urine samples of men were moderately correlated with the CT load of urine samples of men (Pearson $r = 0.61$). Therefore, the results of urine Cq values could be a less stable indication of CT load. This is an expected finding, as we know, concentration in urine can differ interindividually and intraindividually and with time because infected cells from the male urethra may get washed out by first void urine.

We showed that men and women repeatedly infected with CT have a lower urogenital CT load, which was also observed in earlier studies, but only among women.^{6,7} Both studies suggest that partial immunity against CT could play a role. However, further research is needed including longitudinal data, immunological markers, and duration of the CT infection for more evidence. Furthermore, patients with a lower CT load could have a lower impact on transmission of CT. It is likely that patients with initial CT infections may have a higher impact on ongoing transmission than patients repeatedly infected with CT. This could highlight the importance of targeting control measures, such as retesting, to those patients with an initial CT infection.

Repeat anorectal CT infections of women were not associated with a lower CT load, which could be a result of the relatively low number of anorectal repeat infections diagnosed among women ($n = 24$). Among men, however, a substantial number of repeat anorectal infections were diagnosed ($n = 110$), and there was also no association between CT load and repeat infection. If the number of repeat anorectal CT infections in women were larger, we would expect a similar anorectal load of repeat infections as men, because men and women have an equal extragenital load.⁹ Nevertheless, because anorectal infections are common among MSM and women, testing and retesting the anorectal site remain important to enhance CT control. Potentially the partial immunity theory does not hold for anorectal infections, which could be related to treatment (azithromycin vs. doxycycline). Because the treatment effectiveness of doxycycline is higher than azithromycin

in uncomplicated anorectal infections,¹³ it could potentially affect the immune response of the body. However, this is only speculative and further research is needed to assess this pattern.

An earlier study assessed the CT load of repeat infections among African American women and showed a lower urogenital CT load when repeatedly infected with CT (5.6 vs. 4.5 CT/mL; $P = 0.015$).⁶ The authors suggest that the generalizability of the results could be a problem for other populations. However, our results among mainly Western individuals are comparable to this particular study.

In conclusion, men and women repeatedly infected with CT have a lower urogenital bacterial load potentially because of partial immunity against CT. However, repeat anorectal infections of men and women were not associated with a lower CT load, indicating that repeat anorectal infections have a similar impact on transmission and sequelae to that of initial anorectal infections.

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