Repeated *Chlamydia trachomatis* infections are associated with lower bacterial loads

K. Gupta1,*, R. K. Bakshi1,*, B. Van Der Pol1, G. Daniel1, L. Brown1, C. G. Press1, R. Gorwitz2, J. Papp2, J. Y. Lee3 and W. M. Geisler1

1Division of Infectious Diseases, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 2Centers for Disease Control and Prevention, Atlanta, GA, USA and 3Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

*Chlamydia trachomatis* (CT) infections remain highly prevalent. CT reinfection occurs frequently within months after treatment, likely contributing to sustaining the high CT infection prevalence. Sparse studies have suggested CT reinfection is associated with a lower organism load, but it is unclear whether CT load at the time of treatment influences CT reinfection risk. In this study, women presenting for treatment of a positive CT screening test were enrolled, treated and returned for 3- and 6-month follow-up visits. CT organism loads were quantified at each visit. We evaluated for an association of CT bacterial load at initial infection with reinfection risk and investigated factors influencing the CT load at baseline and follow-up in those with CT reinfection. We found no association of initial CT load with reinfection risk. We found a significant decrease in the median log10 CT load from baseline to follow-up in those with reinfection (5.6 CT/ml vs. 4.5 CT/ml; *P* = 0.015). Upon stratification of reinfected subjects based upon presence or absence of a history of CT infections prior to their infection at the baseline visit, we found a significant decline in the CT load from baseline to follow-up (5.7 CT/ml vs. 4.3 CT/ml; *P* = 0.021) exclusively in patients with a history of CT infections prior to our study. Our findings suggest repeated CT infections may lead to possible development of partial immunity against CT.
Reinfection was defined as a positive CT NAAT at the 3- and/ or 6-month visit diagnosis of cervicitis (6.4 (5.1–7.1) vs. 5.7 (4.7–6.8); \( P = 0.06 \)) and BV (6.1 (5.2–7.0) vs. 5.7 (4.6–6.8); \( P = 0.09 \)).

CT infection occurred in a total of 37 (18.5%) participants, with a median time to detection of reinfection of 92 days (range 54–204). Of the 37 reinfected participants, 19 (51.4%) were CT-positive at the 3-month follow-up visit only, 12 (32.4%) at the 6-month follow-up visit only and six (16.2%) at both the 3- and 6-month follow-up visits; participants that were CT-positive at a follow-up visit were provided azithromycin 1 g for CT treatment. There was no association of participant characteristics with CT reinfection. We also found no significant association between baseline CT bacterial load and subsequent reinfection risk: median (IQR) \( \log_{10} \) baseline load: 5.8 (4.8–6.9) vs. 6.2; \( \chi^2 = 0.08 \) and in those with a baseline CT load at baseline and the time of reinfection were evaluated with the Wilcoxon signed-rank test. Associations of participant characteristics with reinfection were evaluated with the Fisher’s exact test. Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC, USA).

Of 239 women that tested positive for CT at enrolment (i.e. the baseline visit), 200 (83.7%) returned for follow-up visits. The study population predominantly consisted of African Americans (95%), with a median age of 22 years (range 16–50). There were 44.8% of women on hormonal contraceptives and 49% were symptomatic. About half of the women (53%) had a history of prior CT infection based on self-report or laboratory test results documentation. Bacterial vaginosis (BV) was the most frequent co-infection at the baseline (22.5%), followed by vulvovaginal candidiasis (14%) and trichomoniasis (8%). The median (IQR) \( \log_{10} \) CT bacterial load at the baseline visit was 5.7 (4.8–6.9) CT/ml. No correlation was found between baseline CT load and age, symptoms, hormonal therapy or prior CT. There was a trend towards a higher baseline CT load (CT/ml) in those who were African American vs. non-African American race (median (IQR) 5.8 (4.8–6.9) vs. 5.0 (4.2–6.2); \( P = 0.08 \)) and in those with a baseline CT bacterial load quantification was performed using the Cobas CT/Neisseria gonorrhoeae assay (Roche Diagnostics, Indianapolis, IN, USA). The assay uses amplification targets on both the CT cryptic plasmid and on the CT genome. To estimate bacterial load, a CT calibrator was run with each testing lot using well-characterised stock CT reference strains with known organism counts (determined in the Van Der Pol laboratory). This allowed creation of cycle threshold standard curves for comparison with clinical samples, providing reliable and reproducible results that allowed for relative quantification on a log scale.

Reinfection was defined as a positive CT NAAT at the 3- and/ or 6-month follow-up visit. The \( \log_{10} \) CT load is presented as median and interquartile range (IQR). The relationship of baseline \( \log_{10} \) CT load with patient characteristics and subsequent reinfection was evaluated with the Kruskal–Wallis test, and
CT/ml in those without reinfection vs. 5.6 (4.7–6.8) CT/ml in those with reinfection (P = 0.44). The findings are consistent with no predictive effect of CT load at the time of treatment on reinfection risk, and do not support our hypothesis that individuals with lower initial CT load prior to treatment have a stronger protective response and are therefore at a lower risk to get reinfected.

We next evaluated the changes in the CT bacterial load between baseline and follow-up in those who had CT reinfection at follow-up. There was a significant decrease in the median (IQR) log10 CT load from the baseline to follow-up visit in women with reinfection (5.6 (4.7–6.8) vs. 4.5 (3.5–6.3) CT/ml; P = 0.015) (Fig. 1a). We found similar differences in CT load between the baseline and follow-up visits upon stratifying the follow-up visits into 3-month (5.4 (4.5–5.8) vs. 4.4 (3.2–5.8); P = 0.078) and 6-month (5.6 (4.8–7.1) vs. 5.55 (3.5–6.6); P = 0.019) visits (Fig. 1b and c). Prior CT infection before the treatment visit did not predict bacterial load at the follow-up visit. There were six (3%) participants found to be CT-infected at both 3- and 6-month visits and their CT loads did not significantly differ between their 3- and 6-month visits.

Next, we investigated whether having had a prior CT infection (before the baseline visit) had an impact on the differences in the CT bacterial load from the baseline to follow-up visit. Upon stratification of women based upon the presence or absence of a CT infection prior to the study, we found that there was a significant decrease in the CT load between visits of reinfected women in only those with a CT infection prior to the baseline visit (5.7 vs. 4.3 CT/ml; P = 0.021), whereas there was no evidence of a change seen in those without any CT infection prior to the baseline visit (5.3 vs. 5.7 CT/ml; P = 0.542) (Fig. 1d). These observations suggest repeated CT infections could lead to the development of partial protective immunity to CT, as reflected in the lower CT loads with subsequent infection.

Timing of CT reinfections likely influences the degree of protective immunity. A prior study showed lower CT reinfection rates when the index (i.e. initial) infection was <6 months vs. more than 6 months earlier [9], indicating that prior CT infections may confer only short-lived partial adaptive immunity in some individuals. This is also consistent with a murine model of genital CT infection that demonstrated insufficient, short-lived adaptive immunity [5]. The lack of development of long-lasting ‘complete’ protective immunity may in part explain why the magnitude of CT bacterial load in an individual did not affect the susceptibility to a subsequent infection, rather it is repeated infections that likely provide some degree of partial immunity that perhaps helps to clear the subsequent infections quicker.

Our study population consisted of only women and was predominantly African American, which may limit the generalisability of our findings. In contrast to our study showing a higher CT bacterial load in African Americans, limited prior studies evaluating the relationship of demographics with CT load have reported a higher CT load in Caucasians compared with African Americans [6]; however, the sample size of Caucasians in our study was very small. We did not know the timing of the prior CT infection in about half of participants, the duration of the CT infection at baseline for all participants, or the timing of reinfection in the affected participants, which may have potentially affected the CT load. Our prior CT infection data were based upon self-reporting and medical record review of laboratory testing results, which may underestimate the proportion of subjects with prior CT infections since all individuals may not be aware of a previous infection or may have been diagnosed with CT infection at another clinic; for those subjects in whom we did have data on prior CT infection, most had the infection more than 6 months prior to baseline. We also cannot rule out the rare possibility that a subject failed their initial treatment and had persisting CT infection rather than reinfection; however, based on a recent randomised controlled CT treatment trial reporting an azithromycin cure rate of 98% in CT-infected women [10], the frequency of treatment failure was likely very low. Our future studies will evaluate how cellular immune responses influence the risk for CT reinfection and will correlate immune response data with CT load.

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