

Delayed Microbial Cure of Lymphogranuloma Venereum Proctitis with Doxycycline Treatment

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Microbial cure of chlamydia proctitis (lymphogranuloma venereum [LGV] and non-LGV) with doxycycline treatment was evaluated by chlamydia DNA and RNA persistence in anal swab specimens. In LGV proctitis, RNA persisted for up to 16 days. In non-LGV chlamydia proctitis, DNA was undetectable after 7 days. These findings support the Centers for Disease Control and Prevention's treatment recommendation of a 21-day doxycycline regimen for LGV proctitis and a 7-day regimen for non-LGV chlamydia proctitis. Delayed microbial cure of LGV proctitis should be considered in improved treatment regimens.

Lymphogranuloma venereum (LGV) is an invasive ulcerative sexually transmitted infection caused by *Chlamydia trachomatis* (chlamydia) biovar L [1]. The infection spreads beyond mucosal linings into connective tissue layers and through lymphatic vessels to loco-regional lymph nodes and causes destructive and systemic inflammatory reactions. In contrast, chlamydia biovars D–K (non-LGV biovars) remain confined to genital and anal mucosa and generally cause few to no symptomatic infections.

LGV can cause an anorectal syndrome characterized by se-

vere proctitis with anal cramps (tenesmus), pain, bloody discharge, and constipation caused by local edema. If left untreated, this can lead to irreversible anal strictures causing soiling, pain, constipation, and megacolon.

LGV is endemic in tropical climate regions [2]. Until 2003, sporadic cases were reported in Western nations, but since 2003, an ongoing epidemic of LGV proctitis among men who have sex with men has emerged, first reported in Rotterdam, The Netherlands, then in other Western countries [3].

It is generally accepted to treat LGV infection with antibiotic regimens of longer duration, compared with the duration used to treat anogenital infections caused by non-LGV chlamydia biovars. The Centers for Disease Control Prevention (CDC) 2006 sexually transmitted disease treatment guidelines recommend a minimum 21-day oral course of doxycycline (100 mg twice daily) for LGV proctitis but only a 7-day course for proctitis caused by non-LGV chlamydia infections [4]. These recommendations are partly based on a meta-analysis by McLean et al. [5]. Nonetheless, the optimal treatment duration for proctitis caused by both LGV and non-LGV biovars has never been studied and is based on clinical experience only, partly because of a lack of criteria by which efficacy can be measured.

In this study, we evaluated the microbial cure of LGV proctitis and non-LGV chlamydia proctitis treated with the CDC standard treatment regimens. Anal swab specimens collected during and after treatment were screened for chlamydia DNA. Traces of bacterial DNA can persist for prolonged periods, even after successful elimination of infectious organisms [6]. In contrast, chlamydia RNA is short lived and can only be produced by live metabolically active chlamydia bacteria [7]. For this reason, additional chlamydia RNA tests were performed for patients with anal swab specimens positive for chlamydia DNA. Treatment was considered successful if no chlamydia DNA or RNA was detected at the end of the doxycycline regimen.

Methods. This study was approved by the Academic Medical Centre Ethical Committee, Amsterdam, The Netherlands. All participants were routinely screened for sexually transmitted infections, including chlamydia and LGV, as described elsewhere [8, 9]. In short, all men reporting receptive anal sex in the previous 6 months were routinely checked for chlamydia proctitis by collection of mucosal swab specimens during anoscopy. Complaints and peri-anal and intra-anal mucous membrane abnormalities were recorded. Gram-stained anal smears were performed, and the number of polymorphous nuclear cells counted per high-power light microscopical field was

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determined. If >10 polymorphous nuclear cells per high-power light microscopical field were counted, patients started with presumptive treatment with doxycycline (100 mg orally twice daily).

Chlamydia proctitis was diagnosed (biovar indiscriminate) within 1 week after screening, when chlamydia DNA was detected by Cobas Amplicor (Roche). Patients with chlamydia proctitis were asked at random for written consent to participate, and additional anal swab specimens for chlamydia diagnostics were collected. If presumptive treatment had not been started 1 week earlier, doxycycline (100 mg orally twice daily) was started. For participants already receiving presumptive treatment, the doxycycline regimen was prolonged. For all patients, biovar-L analysis of chlamydia-positive anal swab specimens was performed, as described elsewhere [10, 11]. If LGV proctitis was excluded, the doxycycline regimen was limited to a minimum of 7 days. If LGV proctitis was diagnosed (by biovar-L analysis) the doxycycline course was prolonged for 21 days. Anal swab specimens were collected at subsequent visits during weeks 1, 2, 3, and 6 (~7, ~14, ~21, and ~42 days, respectively, after the commencement of doxycycline treatment). During this follow-up period, patients were asked to refrain from sexual contact. Swab specimens were eluted in 2 mL of 2SP medium (Roche). For chlamydia DNA-positive samples, biovar-L determination was performed. A second anal swab specimen was stored at -80°C in 2 mL of L6 buffer and was later thawed for RNA extraction. Nucleic acid (DNA-RNA) was isolated, using the commercially available EasyMAG system (Biomerieux), from anal swab specimens positive for chlamydia DNA or when inhibition of the DNA test occurred. An equivalent of 10 μL of the L6 extract was used in an Aptima CT single assay (Genprobe) targeting chlamydia ribosomal RNA by TMA analysis with use of a Tigris DTS analyzer (Genprobe). Participants who missed >1 visit were excluded from the overall study analysis.

Results. Thirty-one male patients with LGV proctitis and 31 with non-LGV chlamydia proctitis were included in the study. Eleven patients with LGV proctitis and 5 with non-LGV chlamydia proctitis missed >1 study visit and were excluded. The remaining 20 patients with LGV proctitis and 26 patients with non-LGV chlamydia proctitis were screened as described in table 1.

One patient with LGV proctitis was chlamydia biovar-L DNA positive and chlamydia RNA positive on day 10 and chlamydia RNA positive (the DNA test was inhibited) on day 16; the patient was chlamydia DNA and RNA negative at the following visit, on day 30. Another patient with LGV proctitis was biovar-L DNA positive and chlamydia RNA positive on day 6 and DNA and RNA negative after 13 days of treatment. Two patients who received a diagnosis of LGV on the inclusion date were chlamydia non-LGV DNA positive until 14 days after the ini-

tiation of treatment. In 1 of these 2 patients, chlamydia RNA was detected on day 7, but both patients were RNA negative after 14 days of treatment. For 2 patients with LGV (at weeks 1 and 2), the internal control of the DNA test showed inhibition of the amplification. Both samples were positive in the RNA test.

At the end of therapy (week 3 visit), LGV proctitis persistent mucous membrane abnormalities were observed in 6 of 16 patients during anoscopic examination. For this reason, doxycycline treatment was prolonged for an additional 21 days for 5 of these 6 patients. None of the 4 patients with LGV proctitis who had persistent chlamydia RNA positivity during treatment had mucous membrane abnormalities at the end of the 21-day regimen.

All but 1 of the patients with non-LGV proctitis were chlamydia DNA negative after 7 days of treatment and remained negative up until the final week 6 visit. The 1 patient was chlamydia non-LGV biovar DNA positive on day 36, after repeated negative chlamydia DNA test results on days 8, 10, and 20.

Discussion. In the present study, we showed persistence of chlamydia RNA in patients with LGV proctitis for up to 16 days during doxycycline treatment. The continued presence of chlamydia RNA suggests the perseverance of metabolically active, infectious pathogens and supports the CDC recommendation for prolonged treatment of LGV treatment for at least 21 days.

In the patients with non-LGV chlamydia proctitis, doxycycline was able to eliminate chlamydia DNA within 7 days, which supports the CDC-recommended shorter doxycycline regimen for treatment of non-LGV chlamydia proctitis, compared with the treatment regimen for LGV proctitis. In 1 patient with non-LGV chlamydia proctitis who tested DNA negative at the week 1, 2, and 3 visits, chlamydia DNA positivity recurred at the week 6 visit. This patient reported unprotected receptive anal sex after the initial treatment, and chlamydia reinfection likely explains the chlamydia DNA recurrence.

The CDC recommends follow-up for patients with LGV until symptoms have disappeared. Some clinicians advise the extension of doxycycline treatment to 42 days if symptoms persist after the initial 21-day regimen. We found no association between delayed microbial cure and persisting mucosal abnormalities; all patients with LGV who had mucosal abnormalities after therapy completion showed microbial cure within the first week. These findings do not support an extended 42-day treatment regimen for LGV proctitis. They do support the immunopathological hypothesis that suggests that LGV disease is caused by a chronic inflammatory process propelled by persisting bacterial peptides long after microbial cure has been achieved [12]. It is doubtful that these inflammatory processes will respond to antimicrobial therapy, but they may require

Table 1. *Chlamydia trachomatis* test results for patients with lymphogranuloma venereum (LGV) proctitis and non-LGV chlamydia proctitis at 5 different time points after the start of treatment.

Visit, week	LGV proctitis ^a					non-LGV chlamydia proctitis ^b				
	No. of patients evaluated ^c	Time from treatment to positive test result, mean days \pm SD	No. of patients positive for chlamydia DNA	Proportion of patients positive for biovar L	No. of patients positive for chlamydia RNA	No. of patients evaluated	Time from treatment to positive test result, mean days \pm SD ^d	No. of patients positive for chlamydia DNA	Proportion of patients positive for biovar L	No. of patients positive for chlamydia RNA
0	20	NA	20	20/20	NA	26	NA	26	0/26	NA
1	17	7.9 \pm 1.5	5	2/5	4	26	7.3 \pm 1.1	0	NA	NA
2	19	14.3 \pm 1.8	2	0/2	1	21	14.1 \pm 1.4	0	NA	NA
3	17	21.1 \pm 1.3	0	NA	NA	24	21.0 \pm 1.1	0	NA	NA
6	19	42.8 \pm 5.3	0	NA	NA	26	45.5 \pm 8.2	1	0/1	1

NOTE. NA, not applicable.

^a Patients with LGV proctitis ($n = 20$) were treated with a 21-day regimen of doxycycline (100 mg orally twice daily).

^b Patients with non-LGV chlamydia proctitis ($n = 26$) were treated with a minimum 7-day regimen of doxycycline (100 mg orally twice daily).

^c Patients from whom swab specimens for *C. trachomatis* tests could be obtained.

^d Time of swab specimen collection was measured from start of doxycycline therapy.

anti-inflammatory approaches, such as corticosteroid therapy. Moreover, doxycycline can cause anal mucosal abnormalities as an adverse effect, which may explain ongoing symptoms after microbial cure of LGV proctitis.

In 3 patients who received a diagnosis of LGV proctitis on the inclusion date, chlamydia DNA of non-LGV biovars persisted during treatment, which suggests that the initial infection included a mixture of different chlamydia biovars that became undetectable at different times. Chlamydia infection caused by >1 biovar could affect diagnostics and requires in-depth investigation. New diagnostic methods that are able to detect multiple biovars in 1 sample could provide important data on the spread of mixed infections at the population level [13, 14].

Under in vitro conditions, *C. trachomatis* can enter a persistent state after exposure to doxycycline [15]. To our knowledge, this is the first human study to show that chlamydia biovar-L organisms are able to survive continued exposure to doxycycline for a longer period than are chlamydia non-LGV biovars. We revealed that prolonged chlamydia-eradicating levels of doxycycline are warranted for successful microbial cure of LGV proctitis. This has implications for improved treatment regimens for LGV infections. A single-dose azithromycin regimen is effective for genital non-LGV chlamydia infections. Some experts recommend 1 g of azithromycin given weekly for 3 weeks for the treatment of LGV, but to date, clinical data for this regimen are lacking [5]. Our data support the necessity of prolonged antichlamydial treatment regimens for LGV proctitis.

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