Single-Dose Azithromycin for Genital Lymphogranuloma Venereum Biovar *Chlamydia trachomatis* Infection in HIV-Infected Women in South Africa: An Observational Study

Peters, Remco P.H. PhD[*,†,‡]; Maduna, Liteboho PhD[*]; Kock, Marleen M. PhD[*,§]; McIntyre, James A. FRCOG[¶,‖]; Klausner, Jeffrey D. MD[**,††]; Medina-Marino, Andrew PhD[*,‡‡]

From the [*]Foundation for Professional Development, Research Unit, East London

[†]Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa

[‡]Department of Medical Microbiology, CAPHRI School of Public Health and Primary Care, Maastricht University Medical Centre, Maastricht, the Netherlands

[§]Tshwane Academic Division, National Health Laboratory Service, Pretoria

[¶]Anova Health Institute, Johannesburg

[‖]School of Public Health and Family, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

[**]David Geffen School of Medicine, Division of Infectious Diseases: Global Health

[††]Fielding School of Public Health, Department of Epidemiology, University of California Los Angeles, Los Angeles, CA

[‡‡]Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa

Correspondence: Remco P.H. Peters, PhD, Foundation for Professional Development, Research Unit, 10 Rochester Rd, Vincent, East London 5217, South Africa. E-mail: remcop@foundation.co.za.

Conflict of Interest and Sources of Funding: The authors do not report any conflict of interest. This work was supported by The Eunice Kennedy Shriver Institute of Child Health and Human Development, National Institutes of Health, award R21HD084274 to A.M.M. and J.D.K., and the President's Emergency Plan for AIDS Relief through the US Agency of the Cooperative Agreement AID 674-A-12-00017 to the Foundation for Professional Development.

Ethical Approval: This study was approved by the University of Pretoria, Faculty of Health Sciences, Pretoria, South Africa (Ref. 401/215) and the University of California Los Angeles (Ref. 15-001351).

ABSTRACT

We conducted an observational study of lymphogranuloma venereum (LGV) biovar *Chlamydia trachomatis* infection in HIV-infected women in South Africa. The LGV biovar was detected in vaginal specimens of 17 (20%) of 85 women with *C. trachomatis* infection; 29% were symptomatic. All cases were negative for the LGV biovar after single-dose azithromycin.

Lymphogranuloma venereum (LGV) biovar *Chlamydia trachomatis* infections occur worldwide. In the United States and Europe, LGV infections predominantly manifest as rectal infection in men who have sex with men.[1] The clinical spectrum of this infection is broad ranging from invasive disease and ulceration to mildly symptomatic and asymptomatic infections.[2] Genital infections in women are occasionally reported but are considered uncommon. In Africa, however, LGV biovar infections classically present as genital ulcer and/or tropical bubo in both men and women.[1]

We recently reported the emergence of genital LGV biovar *C. trachomatis* infection in women in South Africa.[3] In that study, *C. trachomatis* biovar L2 was detected by targeted polymerase chain reaction (PCR) and confirmed by whole-genome sequencing in remnant vaginal swab specimens of a small number of women presenting with vaginal discharge.[3] This observation is concerning because doxycycline is recommended for treatment of LGV biovar infections; however, South Africa's management regimen for vaginal discharge syndrome comprises single-dose azithromycin, ceftriaxone, and metronidazole.[4,5]

Single-dose azithromycin has been proposed for the treatment of LGV biovar *C. trachomatis* infections, but azithromycin's clinical effectiveness is unknown and treatment failure of rectal infections has been reported.[6] A poor response to treatment of genital LGV biovar infections in women could undermine the effectiveness of syndromic management for sexually transmitted infections (STIs). We conducted an observational study using stored specimens to determine the treatment response of LGV biovar *C. trachomatis* infection to single-dose azithromycin in pregnant women living with HIV in South Africa.

METHODS

This study was a subanalysis of a larger prospective cohort study of the acceptability and feasibility of integrating STI screening into antenatal care services in 3 low-income settings in Pretoria, South Africa.[7] In brief, after informed consent, HIV-infected pregnant women attending antenatal care were administered a questionnaire and requested to self-collect 2 vulvovaginal swabs. The first swab was used for detection of *C. trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* using the Xpert CT/NG and TV assays (Cepheid, Sunnyvale, CA); the second swab was stored at −20°C for further microbiological analysis. Single-dose 1 g azithromycin was provided to all women with a positive Xpert test for *C. trachomatis*, and a follow-up visit 3 weeks later was scheduled. Repeat specimen collection and Xpert testing was performed at the follow-up to determine treatment outcome.[8]

Biobanked specimens from women who tested Xpert positive for *C. trachomatis* and corresponding swabs collected during follow-up from those with a positive LGV PCR were included in this study. Presence of *C. trachomatis* DNA was confirmed using the Presto CT/NG PCR assay (Goffin Molecular Diagnostics, Houten, the Netherlands),[9] followed by detection of the LGV biovar using targeted PCR on the LightCycler 480 (Roche Diagnostics, Basel, Switzerland).[10] This LGV PCR is widely used and well validated.[3,11]

RESULTS

Vaginal swab specimens of 85 women diagnosed with *C. trachomatis* infection were identified from a cohort of 427 HIV-infected women attending antenatal care services for the first time in their pregnancy. The median age of these women was 30 years (interquartile range [IQR], 9 years), 36 (42%) were married or living together with a stable partner, 23

(27%) reported condom use during last sex act, and 13 (15%) reported multiple sexual partners. Eighteen (21%) of the 85 women with *C. trachomatis* presented with vaginal discharge; 10 (12%) of 85 had coinfection with *N. gonorrhoeae*, and 31 (36%) of 85 with *T. vaginalis*; none presented with genital ulcer syndrome.

The presence of *C. trachomatis* DNA was confirmed in all 85 vaginal specimens selected, with LGV biovar further detected in 17 (20%) of 85 specimens. There was no difference between the 17 women with LGV biovar compared with the 68 women with non-LGV biovar infection with regard to age (32 ± 9 vs. 30 ± 10 years; $P = 0.71$), having a stable partner relationship (7/16 [44%] vs. 29/68 [43%]; $P = 0.94$), condom use during last sex act (4/16 [25%] vs. 19/68 [28%]; $P = 1.0$), or having multiple sexual partners (4/16 [25%] vs. 9/68 [13%]; $P = 0.26$). Vaginal discharge, but not regional lymphadenopathy, fever, or pelvic pain, was reported by 5 (29%) of 17 women with LGV biovar compared with 13 (19%) of 68 with non-LGV biovar infection ($P = 0.34$); the others were asymptomatic. There was no significant difference in coinfection with *N. gonorrhoeae* (3/17 [18%] vs. 7/68 [10%]; $P = 0.41$) or *T. vaginalis* (6/17 [35%] vs. 25/68 [37%]; $P = 1.0$).

A total of 67 (79%) of 85 women attended their follow-up visit: 11 (65%) of 17 women with LGV biovar infection compared with 56 (84%) of 67 of those with non-LGV biovar infection ($P = 0.18$). There was no difference in follow-up time between these 2 groups (median of 21 [IQR, 21–28] vs. 23 [IQR, 21–28] days; $P = 0.83$). Follow-up LGV biovar PCR was negative in 11 (100%) of 11 (95% confidence interval, 72%–100%) women with LGV biovar infection at baseline. Specifically, at follow-up 8 of 11 tested Xpert negative, whereas 3 (27%) of 11 tested Xpert CT/NG and Presto CT/NG Assay positive but LGV biovar PCR negative (Fig. 1). Among these 3 women, none experienced any symptoms, and none had used condoms during last sex act; one of those women reported having multiple sexual partners, whereas the other 2 reported that they believed their partner had other sexual contacts. There was no statistical difference in *C. trachomatis* Xpert test positivity at follow-up between women with (3/11; 27%) and without (19/56; 34%) LGV biovar infection ($P = 1.0$), respectively.

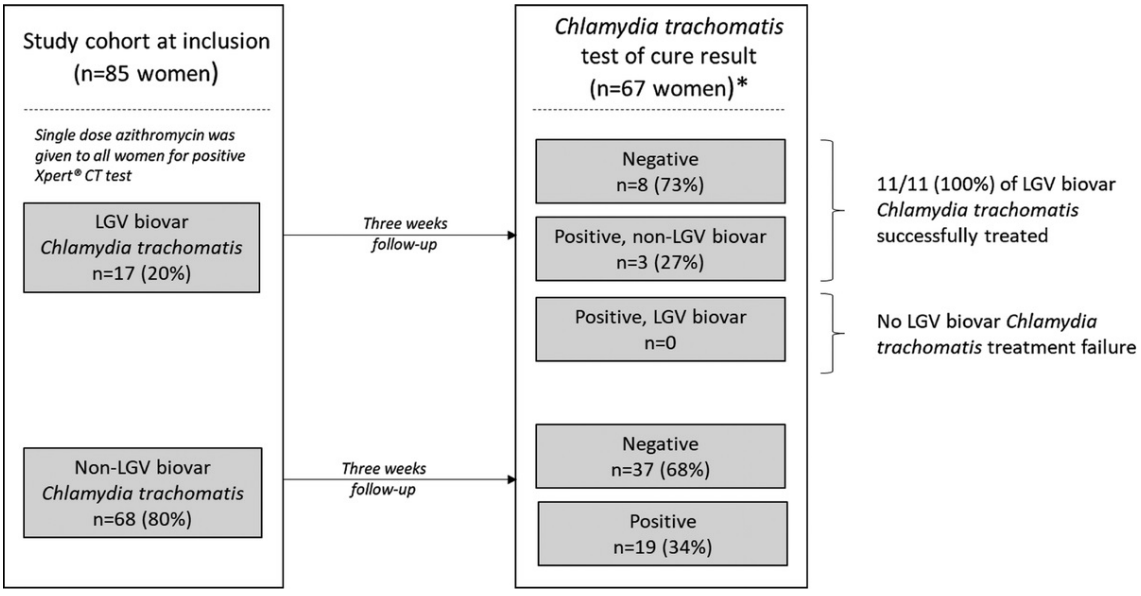

Figure 1: Treatment outcomes of single-dose azithromycin for genital LGV biovar *C. trachomatis* infection in pregnant women living with HIV in South Africa.

DISCUSSION

This study confirms our previous report of the presence of genital LGV biovar *C. trachomatis* infection in women in South Africa.[3] In our current study, surprisingly, 20% of *C. trachomatis* strains belonged to the LGV biovar. The geographical distribution of genital LGV biovar infection is unclear: we did not observe any LGV biovar infections in 2 previous cohorts of women from Mopani District, approximately 300 km away from the current study[3,12]; however, 4 cases were recently identified in a small study at 2 distinct facilities in the Eastern Cape province, >1000 km away (O. Taku, personal communication). We do not have information on the sexual partners of women with LGV biovar infection in any of these studies. Furthermore, about a quarter of the women with LGV biovar infection presented with vaginal discharge; the others were asymptomatic. This is a similar diverse clinical spectrum as reported for rectal infection.[6,13–15] Further research is warranted to determine the epidemiological and clinical characteristics of genital LGV biovar *C. trachomatis* infection in women in South Africa.

All women with genital LGV biovar *C. trachomatis* infection had a negative LGV test result at follow-up, suggesting good effectiveness of single-dose azithromycin. Although these results are encouraging, our study is observational with a relatively small sample size and based on retrospective laboratory testing. Another observational case series reported treatment failure of single-dose azithromycin in 2 of 7 men with rectal LGV biovar infection.[6] Azithromycin efficacy for *C. trachomatis* may be higher for the genital than anorectal site.[16] Although azithromycin in single- or multiple-dose regimens has been suggested for LGV biovar infections, consistent and concluding evidence is lacking to recommend such treatment.[5] A randomized trial would be appropriate to determine the clinical effectiveness of azithromycin for genital LGV biovar infection in women.

However, 3 women with LGV biovar infection at baseline and negative test result for LGV biovar at 3-week follow-up had positive Xpert CT/NG and Presto CT/NG test results at follow-up. It is unclear whether this reflects successful clearance of the LGV biovar or that other factors play a role. The overall *C. trachomatis* cure rate is lower than expected: 19 (34%) of 56 women with non-LGV biovar infection also tested positive for *C. trachomatis* at follow-up.[17] This likely reflects the high incidence of STIs and complexity of managing STIs in our setting where multiple sexual partnerships are common and effective partner notification and treatment cannot be ensured.[8] However, as mixed biovar *C. trachomatis* infections are not common, false-negative LGV PCR results due to difference in assay sensitivity cannot be ruled out.[18,19] As such, it is uncertain whether the LGV biovar infection was cured by single-dose azithromycin in these 3 women.

This study has several limitations. First, our retention was less than 80% despite putting various measures in place for participants to return for their follow-up visit. Our study was observational, and LGV PCR was only performed retrospectively. Because there were no differences between women who did and did not present for the follow-up testing visit, we do not think that loss to follow-up had an impact on our findings. Second, the LGV PCR was performed on a different swab from that of the *C. trachomatis* Xpert test, but laboratory-confirmatory testing confirmed the presence of *C. trachomatis* DNA in all swabs.

In conclusion, this study confirms that genital LGV biovar *C. trachomatis* infection circulates in women in our high-STI-prevalence setting. Furthermore, our observations suggest that these infections may respond well to treatment with single-dose azithromycin. Further studies

are warranted to confirm the clinical effectiveness of single-dose azithromycin for genital LGV biovar infection in women in Africa.

REFERENCES

1. Stoner BP, Cohen SE. Lymphogranuloma venereum 2015: clinical presentation, diagnosis, and treatment. Clin Infect Dis 2015; 61(Suppl 8):S865–S873.
2. Handsfield HH. Lymphogranuloma venereum treatment and terminology. Sex Transm Dis 2018; 45:409–411.
3. Peters RPH, Doyle R, Redelinghuys MJ, et al. *Chlamydia trachomatis* biovar L2 infection in women in South Africa. Emerg Infect Dis 2017; 23:1913–1915.
4. Department of Health of the Republic of South Africa. Sexually transmitted infections management guidelines 2015. Pretoria, South Africa. Available at: https://health-e.org.za/wp-content/uploads/2015/06/STIguidelines3-31-15cmyk.pdf. Accessed January 21, 2019.
5. De Vries HJC, de Barbeyrac B, de Vrieze NHN, et al. 2019 European guideline on the management of lymphogranuloma venereum. J Eur Acad Dermatol Venereol 2019; 33:1821–1828.
6. Leeyaphang C, Ong JJ, Chow EP, et al. Treatment outcomes for rectal lymphogranuloma venereum in men who have sex with men using doxycycline, azithromycin, or both: A review of clinical cases. Sex Transm Dis 2017; 44:245–248.
7. Mudau M, Peters RP, de Vos L, et al. High prevalence of asymptomatic sexually transmitted infections among human immunodeficiency virus-infected pregnant women in a low-income South African community. Int J STD AIDS 2018; 29:324–333.
8. Medina-Marino A, Mudau M, Kojima N, et al. Persistent Chlamydia trachomatis, Neisseria gonorrhoeae and Trichomonas vaginalis positivity after treatment among human immunodeficiency virus–infected pregnant women. Int J STD AIDS 2020; 31:294–302.
9. Cuylaerts V, de Baetselier I, Muvunyi CM, et al. Implementation and evaluation of the Presto combined qualitative real-time PCR assay for Chlamydia trachomatis and Neisseria gonorrhoeae in Rwanda. Afr J Lab Med 2019; 8:739.
10. Morre SA, Spaargaren J, Fennema JS, et al. Real-time polymerase chain reaction to diagnose lymphogranuloma venereum. Emerg Infect Dis 2005; 11:1311–1312.
11. Cole MJ, Field N, Pitt R, et al. Substantial underdiagnosis of lympho-granuloma venereum in men who have sex with men in Europe: Preliminary findings from a multicentre surveillance pilot. Sex Transm Infect 2019; 96:136–142.
12. Peters RP, Dubbink JH, van der Eem L, et al. Cross-sectional study of genital, rectal, and pharyngeal chlamydia and gonorrhoea in women in rural South Africa. Sex Transm Dis 2014; 41:564–569.
13. Simons R, Candfield S, French P, et al. Observed treatment responses to short-course doxycycline therapy for rectal lymphogranuloma venereum in men who have sex with men. Sex Transm Dis 2018; 45:406–408.
14. Saxon C, Hughes G, Ison C, et al. Asymptomatic lymphogranuloma venereum in men who have sex with men, United Kingdom. Emerg Infect Dis 2016; 22:112–116.
15. de Vrieze NHN, van Rooijen M, van der Loeff MFS, et al. Anorectal and inguinal lymphogranuloma venereum among men who have sex with men in Amsterdam, the Netherlands: Trends over time, symptomatology, and concurrent infections. Sex Transm Infect 2013; 89:548–552.

16. Kong FYS, Horner P, Unemo M, et al. Pharmacokinetic considerations regarding the treatment of bacterial sexually transmitted infections with azithromycin: A review. J Antimicrob Chemother 2019; 74:1157–1166.
17. Geisler WM, Uniyal A, Lee JY, et al. Azithromycin versus doxycycline for urogenital Chlamydia trachomatis infection. N Engl J Med 2015; 373:2512–2521.
18. Quint KD, Bom RJ, Quint WG, et al. Anal infections with concomitant Chlamydia trachomatis genotypes among men who have sex with men in Amsterdam, the Netherlands. BMC Infect Dis 2011; 11:63.
19. Versteeg B, Dubbink JH, Bruisten SM, et al. High-resolution multilocus sequence typing reveals novel urogenital Chlamydia trachomatis strains in women in Mopani district, South Africa. Sex Transm Infect 2015; 91:510–512.