

SHORT REPORT

Epidemiology and natural history of ligase chain reaction detected chlamydial and gonococcal infections

D M Kissin, S Holman, H L Minkoff, L DeMeo, W M McCormack, J A DeHovitz

Sex Transm Infect 2002;78:208–209

Objectives: Ligase chain reaction (LCR) technology has dramatically increased the sensitivity of tests for sexually transmitted infections (STIs). It is unknown whether low copy infections (LCR positive, culture negative) have any clinical consequences. We assessed the clinical significance of untreated low copy *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in a cohort of sexually active women.

Methods: We studied a cohort of sexually active women followed at 6 month intervals for up to 3 years. Frozen urine specimens from 181 women with negative cultures for *C trachomatis* and *N gonorrhoeae* who were "high risk" (defined as being less than 40 years old at baseline, and having either *Trichomonas vaginalis* at baseline or a history of more than one sexual partner during the 12 months before baseline) were tested for *C trachomatis* and *N gonorrhoeae* by LCR (Abbott Laboratories, Abbott Park, IL, USA). The specimens from all visits for each person were pooled and LCR was performed on the pool. Laboratory results were linked to clinical information. We also tested all urine samples obtained from patients with a positive culture.

Results: 10 additional infections (nine *C trachomatis* and one *N gonorrhoeae*) were detected with LCR technique. None of the women with low copy infection had evidence of subsequent pelvic inflammatory disease or ectopic pregnancy. Pooling of urine samples resulted in a 47% decline in the number of tests performed.

Conclusions: Additional STIs can be identified when using LCR. Pooling of urine specimens is a cost saving technique for *C trachomatis* and *N gonorrhoeae* testing.

Infections caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are among the most prevalent of all sexually transmitted infections (STIs) and have comprised the largest proportion of all STIs reported to Centers for Disease Control and Prevention.¹ Complications and sequelae of these infections can be severe, including salpingitis with subsequent risk of infertility, ectopic pregnancy, or chronic pelvic pain.^{2–4} According to the Institute of Medicine's recent report, these are the most costly outcomes of any STI except HIV/AIDS.⁵ Up to 70% of sexually active women with chlamydial and gonococcal infections are asymptomatic.

The substantial proportion of asymptomatic patients makes diagnosis of *C trachomatis* and *N gonorrhoeae* infections largely dependent on diagnostic tests. Isolation of the organisms in culture has been the traditional method for laboratory diagnosis. Cultures, however, require technical expertise, rigorous transport conditions to preserve specimen viability and, for chlamydial infections, have a turnaround time of 2–3 days. Nucleic acid amplification tests (NAAT) based on polymerase chain reaction, ligase chain reaction (LCR), and transcription mediated amplification technology are now

commercially available. These tests have good sensitivity and excellent specificity. In addition, these tests offer the advantages of automation, shorter processing times, less demanding transport requirements, and the possibility of using easily obtained specimens, such as urine.

Since NAAT is more sensitive than culture, women who have negative cultures and positive NAAT may be infected with fewer organisms, and thus have milder disease, than women who have positive cultures. The objective of this study was to identify such women (negative culture, positive NAAT) and to assess their clinical status.

METHODS

Study subjects were women who participated in the Heterosexual AIDS Transmission Study (HATS). This cohort has been described in detail elsewhere.⁶ Briefly, between March 1990 and December 1993, 665 HIV negative women (18–50 years of age) with no history of injecting drug use were recruited into a prospective study to assess the risk of heterosexual acquisition of HIV and other STIs. The women had to have had at least one male sexual partner within the previous year and were recruited from medical clinics in central Brooklyn. Follow up visits occurred approximately every 6 months, with an interview, laboratory tests, including cultures for *C trachomatis*, *N gonorrhoeae*, *Trichomonas vaginalis*, and physical examination. Urine samples obtained during baseline and at each subsequent visit were processed and then stored at a NIH repository in Bethesda, Maryland at -70°C .

LCR tests for *C trachomatis* and *N gonorrhoeae* were performed on urine samples obtained at the time of the positive cultures. In order to determine the prevalence of LCR positive/culture negative *C trachomatis* and *N gonorrhoeae* genital infections we tested urine specimens from a subset of 181 women who had consistently negative cultures for these infections and who we defined as "high risk" (less than 40 years old at baseline, and having either *T vaginalis* at baseline or a history of more than one sexual partner during the 12 months before baseline). We utilised the *C trachomatis* and *N gonorrhoeae* LCR assay (Abbott Laboratories, Abbott Park, IL, USA) performed by experienced personnel, who were unaware of the previous culture results. Urine specimens were completely thawed on the day of processing. We pooled urine specimens from all visits for each person (if there were two or more visits) and then performed LCR on the pool. For each pool, a 1 ml aliquot of urine from each visit was transferred to a 15 ml pooling tube. This was then vortexed and 1 ml of the pool was used for processing. The processing and DNA amplification included standard procedures according to the manufacturer's instructions. Pooled samples were evaluated as described in published studies^{7–10} using lowered sample cut-off (S/CO) ratios. Specimens from each positive pool were tested individually.

Analysis of health outcomes included development of pelvic inflammatory disease (PID) and ectopic pregnancy, which were reported or diagnosed during follow up visits. The statistical analysis was conducted with SPSS 10.0 software (SPSS Inc, Chicago, IL, USA).

RESULTS

We tested urine samples obtained simultaneously with endocervical cultures from 58 women who had had positive cultures for *C trachomatis* and nine women who had had positive *N gonorrhoeae* cultures. LCR tests were positive in 49 (84.5%) chlamydia infected women and in six (67%) of nine women who had had gonorrhoea.

Of the 181 "high risk" women whose cultures for *C trachomatis* and *N gonorrhoeae* were negative, 10 (5.5%) had positive urine LCR tests. Nine women had positive LCR tests for chlamydial infection; one woman had a positive LCR for gonorrhoea.

None of the 10 women had evidence of PID during the follow up period, which ranged from 11 to 46 months. By comparison, PID was diagnosed in two of 66 women who had been treated for gonococcal or chlamydial infection and in eight of 171 women who had neither culture, nor LCR test evidence of these infections. None of these differences are statistically significant using χ^2 test for contingency table method. No ectopic pregnancies were noted in any of the above mentioned groups.

We calculated the cost savings of pooling. If pooling was not used, 484 LCR tests would have been done. We actually performed 254 tests, which represents a 47% savings.

DISCUSSION

Archived urine samples proved to be useful for retrospective detection of chlamydial and gonococcal infection. The samples were mostly casual (rather than first void) specimens, and had been frozen for up to 10 years. However, we found chlamydial and gonococcal nucleic acids in 49 (84.5%) of 58 and in six (67%) of nine urine specimens collected from women who had positive cultures, for *C trachomatis* and *N gonorrhoeae*, respectively.

We assumed that LCR positive/culture negative results represent "low copy" *C trachomatis* or *N gonorrhoeae* infections. However, the small number of patients with these infections did not allow us to assess the association of low copy *C trachomatis* or *N gonorrhoeae* infections with PID or ectopic pregnancy. Additional studies involving larger numbers of patients with low copy infections are needed.

Pooling of urine specimens for testing *C trachomatis* and *N gonorrhoeae* has been shown to be accurate and cost effective compared to testing individual samples.⁷⁻¹⁰ Owing to theoretical concern that pooling would dilute the low level positive sample below the limit of detection of the assay we used a lowered S/CO ratio. The lower S/CO ratio allowed us to detect two true chlamydia positive pools, which otherwise would have been considered negative.

Cost savings associated with pooling depend on the size of the pools and prevalence of infections in the population. With the proportion of individuals with detected *C trachomatis* and *N gonorrhoeae* infections during the study being 5.5% we performed 47% fewer tests.

CONCLUSIONS

Additional STIs can be identified when using the LCR test on urine specimens. Pooling of urine specimens is a cost saving technique for *C trachomatis* and *N gonorrhoeae* testing that can be utilised for research purposes.

Key messages

- Association of low copy *C trachomatis* and *N gonorrhoeae* infections with pelvic inflammatory disease and ectopic pregnancy was not revealed.
- Pooling of urine specimens for LCR is a cost saving means to detect STIs.
- Additional STIs can be detected by using LCR test on urine specimens.

ACKNOWLEDGEMENTS

This study was supported in part by National Institutes of Health, Fogarty Award 3 D43 TW00233-05 and Downstate College of Medicine Research Investment Award.

CONTRIBUTORS

DMK was involved in the study design, collected the data, and wrote the paper; SH was involved in the study planning, study sampling, and provided critical reviews of the manuscript; HLM formulated the hypothesis and contributed to the writing of the paper; LDeM performed all laboratory work; WMMcC developed the original idea for the paper, provided suggestions for the analysis; JADeH conceptualized and supervised the study.

Authors' affiliations

D M Kissin, J A DeHovitz, SUNY Albany School of Public Health, NY, USA

S Holman, H L Minkoff, L DeMeo, W M McCormack, J A DeHovitz, SUNY Downstate Medical Center, NY, USA

This paper was presented in part at the International Congress of Sexually Transmitted Infections, Berlin, Germany, 24-27 June 2001.

Correspondence to: Professor Jack A DeHovitz, Department of Preventive Medicine, SUNY Downstate Medical Center, Box 1240-450 Clarkson Avenue, Brooklyn, NY 11203-2098, USA; jdehovitz@netmail.hscbklyn.edu

Accepted for publication 1 March 2002

REFERENCES

- 1 CDC. Summary of notifiable diseases, United States, 1998. *MMWR* 1999;**47**(53).
- 2 Cates W, Rofs KT, Aral SO. Sexually transmitted diseases, pelvic inflammatory disease, and infertility: an epidemiologic update. *Epidemiol Rev* 1990;**12**:199-220.
- 3 Cates W, Wasserheit JN. Genital chlamydial infections: epidemiology and reproductive sequelae. *Am J Obstet Gynecol* 1991;**164**:1771-81.
- 4 Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: impact on human reproduction. *Hum Reprod Update* 1999;**5**:433-47.
- 5 Institute of Medicine. *The hidden epidemic: confronting sexually transmitted diseases*. Washington, DC: National Academy Press, 1997.
- 6 DeHovitz JA, Kelly P, Feldman J, et al. Sexually transmitted diseases, sexual behavior, and cocaine use in inner-city women. *Am J Epidemiol* 1994;**140**:1125-34.
- 7 Krepel J, Patel J, Sproston A, et al. The impact on accuracy and cost of ligase chain reaction testing by pooling urine specimens for the diagnosis of Chlamydia trachomatis infections. *Sex Transm Dis* 1999;**26**:504-7.
- 8 Morre SA, Meijer CJLM, Munk C, et al. Pooling of urine specimens for detection of asymptomatic Chlamydia trachomatis infections by PCR in a low-prevalence population: cost-saving strategy for epidemiological studies and screening programs. *J Clin Microbiol* 2000;**38**:1679-80.
- 9 Kacena KA, Quinn SB, Howell MR, et al. Pooling urine samples for ligase chain reaction screening for genital Chlamydia trachomatis infection in asymptomatic women. *J Clin Microbiol* 1998;**36**:481-5.
- 10 Kacena KA, Quinn SB, Hartman SC, et al. Pooling of urine samples for Neisseria gonorrhoeae by ligase chain reaction: accuracy and application. *J Clin Microbiol* 1998;**36**:3624-8.