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Limits of Chlamydial Diagnostic Tests in Epidemiological Studies

To The Editor—We read the article by Geisler et al [1] with great interest. They demonstrated, using an elegant study design, that the odds of *Chlamydia trachomatis* reinfection 1–12 months after treatment was 4 times that for participants with persistent infection (defined as a positive result of a single test performed 6–47 days after the initial positive sample was collected), compared with participants with spontaneous resolution. The authors based their findings on nucleic acid amplification test (NAAT) results and concluded that treatment for *Chlamydia* infection may attenuate protective immunity in some patients. However, our recent findings have shown that epidemiological data based on results of a single NAAT should be interpreted with much more caution to avoid premature conclusions on *Chlamydia* infection status and further inferences on immunity [2, 3].

By using multiple time sequential sampling performed 3–8 weeks after treatment among patients with *Chlamydia* infection, we demonstrated that *Chlamydia* ribosomal RNA and/or DNA detection was highly intermittent, with low bacterial loads and high rates of detection (ie, 42% of the women with low risk for reinfection tested positive) [2, 3]. These findings may have significant implications for epidemiological studies. When intermittent detection is common after treatment (our study period was limited to 2 months), performance of a single NAAT, which is done in many epidemiological studies, would miss detecting *Chlamydia* in several patients who are intermittently positive. It is unknown whether such outcome misclassification would differ between the 2 exposure groups (ie, patients with persistent infection and those with resolved infection) in the study by Geisler et al. The substantial microbiological detection rate following treatment in our recent studies [2, 3] corresponds with the growing concerns that azithromycin treatment failure rates may be much more common than assumed so far [4]. Therefore, Geisler et al may have falsely attributed reinfection to some patients who tested positive between 1 and 12 months after treatment. Substantial number of their patients indeed showed a low behavioral risk for reinfection (ie, they were either not sexually active, used condoms during intercourse, or had no new sex partner). Such outcome misclassification would likely be differential (ie, dependent on the exposure): a positive NAAT result due to causes other than reinfection (eg, treatment failure) is likely for patients with persistent infection but not likely for patients with spontaneous resolution. If infection in 42% (our observed rate of detection among patients with a low risk for reinfection) of the 31 patients with persistent infection was falsely categorized as reinfection, this would reduce the reinfection rate from 19.9% to 11.5% and would reduce the unadjusted relative risk from 4.4 (95% confidence interval [CI], 1.1–17.6) to 2.5 (95% CI, .6–10.5) for the association between unresolved infection and reinfection. If a smaller percentage, such as 10%, of reinfections were misclassified, the reinfection rate would be 17.9%, and the association would only reach borderline statistical significance (relative risk, 3.9 [95% CI, .98–15.9]). Although it is unknown whether such bias changes during the follow-up period, research could explore whether it would help to exclude test results obtained >1 month after treatment from analyses. Several countries now recommend waiting at least until the third month after treatment to test for reinfection [5].

As is well known, it is complex to determine infection status on the basis of a single NAAT result. A positive NAAT result after treatment may reflect different causes: an established reinfection, transient *Chlamydia* DNA after sex with an infected partner, clinical treatment failure with persistent infection, intermittent microbiological failure, or even resolved infection [2–4, 6]. A single positive NAAT result in itself provides little information about clinical implications, including pathogenicity and severity of infection, and underlying immune mechanisms. Infection with *Chlamydia trachomatis* induces partial immunity at best [7], and the extent to which the highly sensitive NAATs are capable of reflecting the degree of immunity is unknown, as is how immunity induced by natural infection would translate into immunity generated by vaccination. It is well recognized that a vaccine is clearly the way forward in *Chlamydia* control [7, 8]. Animal studies are promising, yet our understanding of chlamydial infection in humans is still limited [9, 10]. Our understanding of the complex life-cycle of the organism, the host immune-responses to infection, and how these relate to clinical implications is necessary to develop vaccines and evaluate their effects on a population. Epidemiological studies are no doubt essential to making the much-desired next steps into a vaccine era, but they need to acknowledge the limits of current state-of-the-art *Chlamydia* diagnostic tests. Taking into account immune

markers [10] and, for example, bacterial load measurements would greatly help, as would the use of promising innovative techniques such as the assessment of viable *Chlamydia* organisms [3]. Such developments are urgently needed when approaching a *Chlamydia* vaccine era.

Notes

Acknowledgments. We thank Prof Dr Servaas A Morré (Maastricht University) for involvement in and critical reflection about this study.

Financial support. This work was supported by the Dutch National Institute for Environment and Health, Center for Infectious Diseases Control (grant 3900051587).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Received 22 November 2013; accepted 6 January 2014; electronically published 9 January 2014.

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The Journal of Infectious Diseases 2014;209:2017–8

DOI: 10.1093/infdis/jiu011