The Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* Biovar 2 in Postgonococcal Urethritis

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(See the editorial commentary by Manhart et al. on pages 872–4)

**Background.** There are few studies on coinfection with genital mycoplasmas and ureaplasmas in men with gonococcal urethritis (GU). The role of these species in postgonococcal urethritis (PGU) is poorly understood. Thus, we conducted a study to determine the prevalence of coinfection with genital mycoplasmas and ureaplasmas among men with GU and to assess the role of these pathogens in PGU.

**Methods.** Three hundred ninety men infected with culture-confirmed *Neisseria gonorrhoeae* participated in the study. *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum* biovar 1, and *Ureaplasma urealyticum* biovar 2 in first-voided urine samples were detected by polymerase chain reaction–based assay at the patients’ initial visits. PGU was judged to be present if the urethral smear was positive for polymorphonuclear leucocytes 7–14 days after treatment for gonorrhea. The association between each microorganism and PGU, measured by the odds ratio, was estimated by multivariate logistic regression analysis.

**Results.** *C. trachomatis*, *M. genitalium*, *M. hominis*, *U. parvum* biovar 1, and *U. urealyticum* biovar 2 were detected in 85 (21.8%), 16 (4.1%), 8 (2.1%), and 33 men (8.5%), respectively. In patients with chlamydia-negative GU, coinfection with *M. genitalium* was associated with a 14.54-fold greater risk of PGU (95% confidence interval, 2.91–72.74), and coinfection with *U. urealyticum* biovar 2 was associated with a 3.64-fold greater risk of PGU (95% confidence interval, 1.24–10.63).

**Conclusions.** Coinfection with *M. genitalium* or *U. ureaplasma* biovar 2 in men with GU was significantly associated with PGU, independent of *C. trachomatis*. Men with GU should be treated presumptively with antibiotics that are active against *C. trachomatis*, *M. genitalium*, and *U. urealyticum* biovar 2.

Many studies have shown that some men with gonococcal urethritis (GU) are coinfected with *Chlamydia trachomatis* [1]. In clinical practice, a presumptive diagnosis of GU is made on the basis of the presence of gram-negative diplococci in urethral smear specimens; treatment for gonorrhea is then initiated. Because a highly sensitive point-of-care test for *C. trachomatis* is not available, treatment decisions must be made before test results are obtained. In addition, many patients treated for GU do not return for their test results, even though testing for *C. trachomatis* has been performed. Although estimates of chlamydial infection in men with GU vary widely, ranging from 4% to 50% [1, 2], the fact that some men with GU are coinfected with *C. trachomatis* has led to the recommendation that men treated for GU should be treated routinely with a regimen that is effective against chlamydial infection [3].

*Trichomonas vaginalis* is another pathogen that causes symptomatic or asymptomatic urethritis in men [4]. Herpes simplex virus and adenovirus are also thought to be associated with nongonococcal urethritis (NGU) [5, 6]. Controversy remains, however, over the significance of these organisms in men with NGU. Conversely, the detection of *Mycoplasma genitalium* by PCR became possible in the 1990s [7, 8], and the results of many studies have suggested that *M. genitalium* causes NGU [9]. Results of our recent study, which specifically...
identified Ureaplasma parvum biovar 1 and Ureaplasma urealyticum biovar 2 in cases of NGU, suggest that U. urealyticum biovar 2 is significantly associated with NGU [10]. M. genitalium and U. urealyticum biovar 2 could also be causes of the urethritis that occurs after treatment of GU with penicillin or cephalosporin antibiotics, because, like C. trachomatis, these pathogens are not susceptible to β-lactam antibiotics. In contrast to the many studies on coinfection with C. trachomatis in men with GU [1, 2], there are few studies on coinfection with genital mycoplasmas and ureaplasmas; their role in postgonococcal urethritis (PGU) is poorly understood.

The purpose of this study was to clarify the association of genital mycoplasmas and ureaplasmas with PGU. We examined first-voided urine samples from 390 men with GU for the presence of C. trachomatis and genital mycoplasmas, including M. genitalium, Mycoplasma hominis, U. parvum, and U. urealyticum, using PCR-based assays to determine the prevalence of coinfection with C. trachomatis, genital mycoplasmas, and genital ureaplasmas among men with GU. We treated gonorrhea with cephalosporin or spectinomycin and assessed the role of these mycoplasmas and ureaplasmas in PGU.

METHODS

A total of 390 men who visited the Department of Urology at Toyota Memorial Hospital (Toyota, Japan) from November 1999 through December 2005 for treatment of GU were enrolled in the study. Some of these patients were included in our previously reported studies [10–12]. The patients ranged in age from 16 to 65 years (mean age, 24 years), and all were Japanese. All of the patients had sexual intercourse with a female partner before visiting the clinic, and they were thought to be heterosexual, although their sexual orientation was not confirmed. The patients provided informed consent for their participation. All of the patients had symptoms and signs that were compatible with acute urethritis, including discharge and ≥5 polymorphonuclear leukocytes (PMNLs) per high-power microscopic field (× 1000) in a Gram-stained urethral smear specimen. On presentation to our clinic, gram-negative intracellular diplococci were observed in the urethral smear specimens, and N. gonorrhoeae was isolated from the urethra by culture.

To detect C. trachomatis, genital mycoplasmas, and genital ureaplasmas, first-voided urine samples were obtained from the patients. A portion of each sample was subjected to a PCR-based assay (Amplicor STD-1 Assay; Roche Diagnostics) for detection of C. trachomatis. For samples obtained from November 1999 through July 2000, another portion was examined for the presence of M. genitalium, M. hominis, U. parvum biovar 1, and U. urealyticum biovar 2 using a PCR- and phylogeny-based assay [11]. From August 2000 through December 2005, mycoplasmas and ureaplasmas were detected by a PCR-microtiter plate hybridization assay [12]. Because performance of these assays was comparable [12], patients whose first-voided urine samples were examined for the genital mycoplasmas and ureaplasmas by either of these 2 assays were included together in this study.

GU was treated with cefixime (268 patients), ceftriaxone (36 patients), or spectinomycin (86 patients). We instructed patients to practice sexual abstinence during treatment and asked them to return for reexamination 7 days later, regardless of the presence or absence of symptoms. We also told them that their sex partners should be examined for genital infection, but we did not follow up with respect to partner management. At the second visit, urethral smear specimens were examined for PMNLs and gram-negative intracellular diplococci, and urethral swab specimens were cultured for N. gonorrhoeae. The urethral smear results were considered to be positive if the specimens contained ≥5 PMNLs per high-power field. At the second visit, PGU was considered to be present if the urethral smear specimen was positive for PMNLs 7–14 days after treatment for gonorrhea in patients in whom gram-negative intracellular diplococci were not observed in urethral smear specimens and whose results of culture for N. gonorrhoeae were negative.

The association between each microorganism and PGU was assessed by the Pearson χ² test or Fisher’s exact test. All comparisons were performed as 2-tailed tests, with statistical significance set at P < .05. Association, measured by OR, was estimated by means of multivariate logistic regression analysis (StatView, version 5.0; SAS Institute).

RESULTS

C. trachomatis, genital mycoplasmas, and genital ureaplasmas detected in patients with GU are summarized in table 1. C. trachomatis, genital mycoplasmas, and/or genital ureaplasmas were detected in 132 (33.8%) of the 390 patients with culture-confirmed N. gonorrhoeae (95% CI, 29.3%–38.4%). C. trachomatis alone or with genital mycoplasmas was detected in 85 patients (21.8%; 95% CI, 17.8%–25.8%). One or 2 species of genital mycoplasma and ureaplasma species were detected in 15 patients (3.8%) coinfected with C. trachomatis (95% CI, 2.0%–5.7%) and in 47 patients (12.1%) who did not have chlamydia coinfection (95% CI, 8.9%–15.2%). Among the 47 patients without chlamydia coinfection, M. genitalium and U. urealyticum biovar 2 were detected in 12 and 23 patients, respectively.

Three hundred twenty-seven patients (83.8%) returned to the clinic after treatment for gonorrhea (table 1). This follow-up rate was high enough to examine the association of C. trachomatis, genital mycoplasmas, or genital ureaplasmas with PGU. Thirty-six patients who were culture-positive for N. gonorrhoeae were excluded from the analysis of the presence of...
PGU. In the remaining 291 patients, eradication of *N. gonorrhoeae* was confirmed by culture after treatment. Of these 291 patients, 104 (35.7%) were shown to have PMNLs in their urethral smear specimens and were considered to have PGU (95% CI, 31.1%–40.4%), including 41 patients who did not have urethritis symptoms. PGU occurred in 51 (77.3%) of 66 patients with chlamydia-positive GU (95% CI, 73.2%–81.3%), including mycoplasma- and/or ureaplasma-positive GU. PGU also occurred in 15 (50.0%) of 30 patients with chlamydia-negative but mycoplasma- and/or ureaplasma-positive GU. PGU was observed in 41 patients who did not have urethritis symptoms. PGU occurred in 51 (77.3%) of 66 patients with chlamydia-positive GU (95% CI, 73.2%–81.3%), including 41 patients who did not have urethritis symptoms. PGU occurred in 51 (77.3%) of 66 patients with chlamydia-positive GU (95% CI, 73.2%–81.3%), including 41 patients who did not have urethritis symptoms. PGU occurred in 51 (77.3%) of 66 patients with chlamydia-positive GU (95% CI, 73.2%–81.3%), including 41 patients who did not have urethritis symptoms.

In the 291 patients who returned to the clinic, *C. trachomatis*, *M. genitalium*, and *U. urealyticum* coinfection was associated with PGU, whereas this mycoplasma was isolated from 2 patients (10.5%) of 25 patients with GU, respectively. In France, Janier et al. [16] detected the mycoplasma in 1 (4.0%) of 25 patients with GU. In Japan, Uno et al. [17] tested 45 patients with GU and detected the mycoplasma in 2 patients (4.4%). Recent *M. genitalium* coinfection rates among men with GU in Africa are reported to be 6%–11% [18, 19], and the rate reported in the United States is 14% [20]. In our present study, involving 390 patients with GU, only 12 (4.1%) were positive for *M. genitalium*, although 85 (21.8%) were positive for *C. trachomatis*. In our previous study, the prevalence of *M. genitalium* was 12.6% among men with NGU and 1.4% among men without urethritis [10]. These findings suggest that the prevalence of *M. genitalium* in men with GU is likely to be low and significantly less than that of *C. trachomatis*, and that the prevalence may be lower than that in men with NGU but higher than that in men without urethritis.

The prevalence of *M. hominis* was 2.1% among men with GU in our present study, whereas this mycoplasma was isolated...
By multivariate logistic regression analysis, *M. genitalium* men coinfected with mycoplasmas and/or ureaplasmas. In particular, ureaplasmas [26, 28]. PGU was observed in 77.3% of men assessed by the Pearson \( x^2 \) test or Fisher’s exact test.

Table 2. Univariate analysis of the association between coinfecting organisms and postgonococcal urethritis (PGU) in 291 men with gonococcal urethritis in whom the eradication of *Neisseria gonorrhoeae* was confirmed by culture after treatment.

<table>
<thead>
<tr>
<th>Coinfecting organism</th>
<th>With PGU (n = 103)</th>
<th>Without PGU (n = 188)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlamydia trachomatis</em></td>
<td>51 (49.0)</td>
<td>15 (8.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><em>Mycoplasma genitalium</em></td>
<td>10 (9.6)</td>
<td>3 (1.6)</td>
<td>.002</td>
</tr>
<tr>
<td><em>Mycoplasma hominis</em></td>
<td>3 (2.9)</td>
<td>1 (0.5)</td>
<td>.132</td>
</tr>
<tr>
<td>Ureaplasma parvum biovar 1</td>
<td>2 (1.9)</td>
<td>4 (2.1)</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Ureaplasma urealyticum biovar 2</td>
<td>13 (12.5)</td>
<td>10 (5.3)</td>
<td>.030</td>
</tr>
</tbody>
</table>

NOTE. The association between each microorganism and PGU was assessed by the Pearson \( x^2 \) test or Fisher’s exact test.

in 6%–12% of men with GU in earlier studies [21–23]. In our previous study, *M. hominis* was detected in 2.8% of men with NGU and in 2.1% of asymptomatic men [10]. There is no apparent difference in the prevalence of *M. hominis* between men with urethritis, regardless of GU or NGU, and men without urethritis.

*U. parvum* biovar 1 and *U. urealyticum* biovar 2 were detected in 2.1% and 8.5% of men with GU, respectively. The coinfection rate of the 2 ureaplasmas with *N. gonorrhoeae* was 10.6%, whereas, in earlier studies in which the 2 ureaplasma species were not yet identified, coinfection rates of ureaplasmas with *N. gonorrhoeae* were reported to be 27%–38% [21–24]. The prevalence of the ureaplasmas among men with GU was lower than that in the previous studies. The prevalence of mycoplasmas and ureaplasmas could depend on many factors, including geographic region, time of investigation, method of detection of the mycoplasmas and ureaplasmas, and sexual behavior of the patients.

*Chlamydia trachomatis* is a cause of urethritis that occurs after treatment of GU with penicillin and cephaporolin antibiotics [1]. Development of PGU, however, was noted also in a large proportion of men with chlamydial infection [25, 26]. Furthermore, the addition of doxycycline to the treatment of GU resulted in a significant reduction in the development of PGU [23]. Pathogens that are susceptible to doxycycline are thought to cause PGU. Mycoplasmas and ureaplasmas lack a cell wall; thus, they are insensitive to penicillins or cephalosporins but susceptible to tetracyclines [27]. In the present study, men with GU were treated with cephaporolin or spectinomycin, each of which was less active against *C. trachomatis*, mycoplasmas, and ureaplasmas [26, 28]. PGU was observed in 77.3% of men coinfected with *C. trachomatis*. PGU occurred in 50.0% of men coinfected with mycoplasmas and/or ureaplasmas. In particular, 6 of 8 men coinfected with *M. genitalium* alone and 6 of 14 men coinfected with *U. urealyticum* biovar 2 alone had PGU. By multivariate logistic regression analysis, *M. genitalium* and *U. urealyticum* biovar 2 were significantly associated with PGU, independent of *C. trachomatis*. This is, to our knowledge, the first study to suggest that *M. genitalium* and *U. urealyticum* biovar 2 may play a pathogenic role in PGU. In the present study, the high follow-up rate (83.8%) after treatment for GU adds to the validity of these findings. In early studies in which the 2 ureaplasmas were not identified, however, *U. urealyticum* biovar 2 was not associated with PGU [23, 24]. In our previous study, *U. urealyticum* biovar 2 was detected more often than *U. parvum* biovar 1 in men with GU or NGU [10]. Conversely, *U. urealyticum* biovar 2 was less often detected than *U. parvum* biovar 1 in asymptomatic men. A significant association was observed between *U. urealyticum* biovar 2 and NGU, but the presence of *U. parvum* biovar 1 in the male urethra might be because of colonization and is therefore not likely to be significant in the development of NGU. In our previous study, when *U. parvum* biovar 1 and *U. urealyticum* biovar 2 were considered together, the ureaplasmas were not significantly associated with urethritis, NGU, or nonchlamydial NGU [10]. These findings might explain why, when *U. parvum* biovar 1 and *U. urealyticum* biovar 2 were not distinguished from each other at the time of detection, no significant association between the ureaplasma and PGU was observed in the earlier studies [23, 24].

In the guidelines published by the Centers for Disease Control and Prevention [3], it has been recommended that men treated for GU be treated routinely with a regimen that is effective against chlamydial infection if they are at high risk for not returning to the clinic or if they are not tested for chlamydial infection by nucleic acid amplification tests. In the Japanese guidelines, however, dual therapy for gonorrhea and chlamydial infection has not been recommended [29]. There are arguments against dual therapy. In Japan, the prevalence of coinfection with *C. trachomatis* in patients with GU is ∼20%, as shown in this study. In Japan, the emergence and spread of clinical isolates of *N. gonorrhoeae* with multidrug resistance,
including fluoroquinolone and oral cephalosporin resistance, has been observed [30]. Therefore, we have always told our patients with GU to revisit our clinic after treatment, emphasizing the risk of occurrence of PGU and of treatment failure. More than 80% of our patients have revisited our clinic for a test-of-cure. We prefer a highly sensitive test for chlamydia rather than treating chlamydial infection presumptively. In addition to testing for C. trachomatis, we have also tested men with gonorrhea for genital mycoplasmas and ureaplasmas at the time of treatment for gonorrhea. We have treated only those men who return for their test results and are positive for C. trachomatis, M. genitalium, and/or U. urealyticum biovar 2. In general, however, follow-up rates after treatment for GU in Japan would not be as high as ours. Many patients treated for GU do not return for a test-of-cure or for results of C. trachomatis testing.

In the guidelines published by the Centers for Disease Control and Prevention in 2006 [3], it is recommended that, because of the high sensitivity of nucleic acid amplification tests for chlamydial infection, patients with a negative chlamydial nucleic acid amplification test result at the time of treatment for gonorrhea do not need to be treated for chlamydia. In the present study, rates of coinfection with M. genitalium (4.1%) and U. urealyticum biovar 2 (8.5%) among men with GU were shown to be low, compared with the C. trachomatis coinfection rate (21.2%). However, when men with chlamydia-negative GU were coinfected with genital mycoplasmas, particularly M. genitalium and U. urealyticum biovar 2, they suffered from mycoplasma- or ureaplasma-associated PGU; when they were not treated for mycoplasma or ureaplasma infection, they could transmit M. genitalium and U. urealyticum biovar 2 to female partners. In women, M. genitalium could be associated with mucopurulent cervicitis, pelvic inflammatory disease, and tubular factor infertility [31]. Ureaplasmas could be associated with chorioamnionitis, spontaneous abortion, preterm birth, low birth weight, and postpartum fever [31]. Thus, men with chlamydia-negative GU should be treated presumptively with antimicrobial agents that are active against M. genitalium and U. urealyticum biovar 2. In clinical settings, point-of-care testing for C. trachomatis at the time of treatment for GU is difficult, and no sensitive and rapid tests for detection of genital mycoplasmas and ureaplasmas are commercially available. In addition, many patients treated for GU do not return for a test-of-cure. Thus, men with GU should be treated presumptively with antimicrobial agents that are active against C. trachomatis, M. genitalium, and U. urealyticum biovar 2. Most therapies for chlamydial infection appear to be effective against M. genitalium infection, as well [9]. However, clinical data on treatment for M. genitalium infection are very limited. Treatment with a 7-day regimen of doxycycline (200 mg/day) resulted in microbiological cure in 94% of patients with M. genitalium-positive NGU [32], whereas a doxycycline regimen of 200 mg/day on the first day and 100 mg/day for the following 13 days was not as effective (79%) [33]. In our previous studies on treatment for M. genitalium-positive NGU with fluoroquinolones, the microbiologic eradication rate of the mycoplasma with a 14-day regimen of levofloxacin (300 mg/day) was 50%, but the eradication rate with a 7-day regimen of gatifloxin (400 mg/day) was 92% [34]. The microbiologic eradication rate of M. genitalium with a single 1-g dose of azithromycin was reported to be 82% [32]. Recently, however, treatment failure with a single 1-g dose of azithromycin was associated with reduced susceptibility of M. genitalium to azithromycin [35]. Most recently, rifalazil, which is effective against chlamydial NGU, was reported to be ineffective in eradicating M. genitalium and U. ureaplasma [36]. There has been controversy over which agents are optimal for M. genitalium infection.

We conclude that coinfection with M. genitalium or U. ureaplasmabiovar 2 in men with GU is associated with PGU, independent of C. trachomatis. However, the study was limited by the small number of patients with GU who were coinfected with genital mycoplasmas and ureaplasmas and by the absence of testing for other coinfecting microorganisms. Several points remain to be elucidated, including which agents should be prescribed for cotreatment of patients with GU and what other coinfecting microorganisms could cause PGU in men with C. trachomatis-, mycoplasma-, and ureaplasma-negative GU. Further studies are needed, but our present results may be informative for preventing PGU in patients with GU.

### Table 4. Multivariate logistic regression analysis of the association between coinfecting organisms and postgonococcal urethritis.

<table>
<thead>
<tr>
<th>Coinfecting organism</th>
<th>All patients (n = 291)</th>
<th>Chlamydia trachomatis-negative patients (n = 225)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. genitalium</td>
<td>OR (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>11.40 (5.86–22.20)</td>
<td>14.54 (2.91–72.74)</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>8.35 (2.07–33.71)</td>
<td>2.50 (0.92–6.72)</td>
</tr>
<tr>
<td>Ureaplasma urealyticum biovar 2</td>
<td>2.50 (0.92–6.72)</td>
<td>3.64 (1.24–10.63)</td>
</tr>
</tbody>
</table>

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References


