Role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in Acute and Chronic Nongonococcal Urethritis

Patrick Horner,1,2 Brenda Thomas,1 Claire B. Gilroy,1 Matthias Egger,3 and David Taylor-Robinson1

1Genitourinary Medicine Section, Department of Medicine (Division A), Imperial College School of Medicine, St. Mary’s Hospital, Paddington, London; 2Department of Genitourinary Medicine, Bristol Royal Infirmary, and 3MRC Health Services Research Collaboration, Department of Social Medicine, University of Bristol, Bristol, United Kingdom

One hundred fourteen heterosexual men with acute nongonococcal urethritis (NGU) and 64 patients without NGU were studied. We determined that *Chlamydia trachomatis* and *Mycoplasma genitalium* were strongly associated with acute NGU after controlling, by means of multivariate analysis, for age, race, sexual lifestyle, and coinfection (odds ratio [OR], 13.0, 95% confidence interval [CI], 2.6–64.5; and OR, 17.9, 95% CI, 2.0–160, respectively). Eighty-six men with acute NGU reattended at least once 10–92 days after treatment; 59 (69%) of these 86 men had urethritis. Seven men had *M. genitalium* detected during 10–92 days of follow-up, and all had urethritis. Ureaplasmas were not associated with acute NGU in multivariate analysis, but their detection was associated with the presence of urethritis during follow-up (P < .014). Ureaplasmas or *M. genitalium* were associated with both chronic NGU, which was defined as urethritis that occurred 30–92 days after the commencement of treatment (P = .028), and chronic NGU with symptoms or signs (P = .005).

Nongonococcal urethritis (NGU) is the most common condition diagnosed and treated in men who attend departments of genitourinary medicine in the United Kingdom [1]. In the United States, it has been estimated that there are 2 million cases per year [2]. Despite intensive microbiological investigation of patients with acute NGU, only *Chlamydia trachomatis* has been firmly established as an etiologic agent that accounts for 30%–50% of cases. *Ureaplasma urealyticum* organisms (ureaplasmas) have long been implicated as a cause of acute NGU, but the evidence is conflicting [3]. Nevertheless, it is generally believed that ureaplasmas cause urethritis, although for what proportion of cases of NGU they might be responsible is unclear and still a matter of speculation [4]. We have demonstrated a significant association of *Mycoplasma genitalium* with acute NGU, which was independent of the presence of *C. trachomatis* and could not be explained by differences in age, race, or sexual lifestyle [5]. Similar observations were made in Denmark [6] and subsequently in Japan [7], France [8], and the United States [9, 10]. This microorganism might account for 15%–25% of cases of acute NGU.

Despite appropriate antibiotic therapy, 20%–60% of patients with acute NGU will have persistent or recurrent urethritis after 1–2 weeks of treatment [11–15]. The presence of urethritis after treatment of acute NGU (often referred to as “chronic NGU”) is one of the most complicated clinical problems in genitourinary medicine, and despite considerable investigation, its etiology is largely unknown and probably multifactorial [11, 14, 15]. Age, race, duration of symptoms, history of NGU, and composition of treatment (a tetracycline or eryth-
romycin) do not appear to predict its development [14]. Some investigators have concluded that chlamydia-positive men respond better to antibiotic therapy than do chlamydia-negative men, whereas others have found no difference, and the reverse has been observed in 2 studies [13, 14]. The reason for this is unclear, but it may reflect a variation in the sensitivity of tests used to identify urethritis at follow-up [13].

Indeed, there is no precise definition of “chronic NGU,” which may explain, at least in part, the wide variation in the reported prevalence of urethritis after treatment. Therefore, it is not surprising that both the etiology and definition of this condition have been identified as subjects in need of major research in the United States [16]. Understanding of this condition has been hampered further by ignorance of the cause of acute NGU in a considerable proportion of patients. It has been speculated that persistent chlamydial infection may account for chronic inflammation in some of the patients. However, C. trachomatis has been identified only rarely during follow-up of men who were treated for acute NGU [11–15]. Both ureaplasmas and M. genitalium have been associated with urethritis at follow-up [12, 17], but only ureaplasmas have been studied extensively by means of culture, and they have been found in only a minority of cases [12]. We demonstrated a significant association of chlamydial hsp60 antibody with the development of urethritis, after treatment of acute NGU, in both chlamydia-positive and chlamydia-negative men, a result that is consistent with the hypothesis that an immune response to hsp60 may be important in the development of at least a proportion of cases of chronic disease [13].

To investigate further the etiology of acute NGU, its natural history after treatment, and the etiology of urethritis after treatment, we undertook a cross-sectional study of men with and without NGU, followed by a prospective follow-up study, over a 3-month period, of men with acute NGU. Here, we present data on the natural history of acute NGU after treatment, with particular reference to symptoms and signs, and on the association of M. genitalium and ureaplasmas with both acute NGU and urethritis after treatment.

**PATIENTS, MATERIALS, AND METHODS**

**Patients.** Men attending the Jefferiss Wing at St. Mary’s Hospital, Paddington, London, for a sexual health assessment were eligible for inclusion into the study. One author (P. H.) recruited the majority of the case patients and control patients, with assistance from other physicians working within the department. Initially, only case patients were recruited. To be included in the study, they had to be clinically symptomatic [5] (i.e., either they complained of a discharge, dysuria, penile irritation, or genital discomfort, or they had urethral discharge on examination after an urethral massage) and had to have NGU present on microscopic evaluation. NGU was diagnosed at initial presentation if there were ≥5 polymorphonuclear (PMN) leukocytes per high-power (×1000) microscope field (HPF) in ≥5 fields of a Gram-stained urethral smear. Control subjects were patients who were attending for a check-up, who were clinically asymptomatic [5] (i.e., they did not complain of a discharge, dysuria, penile irritation, or genital discomfort, and they had no urethral discharge on examination after urethral massage), and had no urethritis on microscopic evaluation. This was defined as <5 PMN leukocytes/HPF in a Gram-stained urethral smear and no threads in 15–20 mL of a first-pass urine (FPU) specimen, or threads that contained <10 PMN leukocytes/HPF in a Gram-stained smear. Men who had taken antibiotics that are known to be active against C. trachomatis, M. genitalium, or both during the previous month (i.e., a tetracycline, a macrolide, or a quinolone) and men who had had an episode of NGU in the 3 months prior to the study period were excluded. Men from whom Neisseria gonorrhoeae was cultured were also excluded, as were those who had a confirmed bacterial urinary tract infection or herpes simplex virus infection.

**Study design.** A total of 114 heterosexual men with acute clinically symptomatic NGU and 64 clinically asymptomatic heterosexual men without NGU were studied, as described elsewhere [5, 13]. Patients were asked to complete a questionnaire requesting details about discharge, dysuria, and penile irritation. In addition, the physician recorded, also on a standardized questionnaire, the patient’s age, race, number of sexual partners in the 3 months prior to the study period, duration of relationships with sexual partners in the 3 months prior to the study period, number of partners during the patient’s lifetime, and whether a discharge was present after urethral massage (table 1). Information on race and number of lifetime sexual partners was incomplete; race was not included in the original questionnaire but was included shortly after the study began. Five (4%) of 114 men with NGU declined to give information on lifetime number of sexual partners, compared with 27 (42%) of 64 men without urethritis (P<.01).

A 5-mm plastic loop was used to prepare a Gram-stained smear and inoculate an agar plate for the isolation of N. gonorrhoeae. A nasopharyngeal swab (Medical Wire and Equipment) was then passed 2–4 cm into the urethra and rolled on a MicroTrak slide (Syva). A FPU specimen was collected subsequently. Patients with acute NGU were treated with doxycycline, 200 mg given initially and then 100 mg per day for 13 days, or enteric-coated erythromycin, 500 mg given 4 times per day for 14 days, if they were intolerant of doxycycline. They were advised to abstain from sexual intercourse, and they were advised that their partner or partners needed to receive treatment at a department of genitourinary medicine.

A total of 109 men with acute NGU were asked to reattend for clinical examination 2 weeks, 6 weeks, and 12 weeks after...
Table 1. Comparison of demographic and sexual lifestyle characteristics and detection of *Chlamydia trachomatis*, *Mycoplasma genitalium*, and ureaplasmas in men with or without acute nongonococcal urethritis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with urethritis (n = 114)</th>
<th>Patients without urethritis (n = 64)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean y ± SD</td>
<td>31.3 ± 8.1</td>
<td>31.1 ± 8.3</td>
<td>.89</td>
</tr>
<tr>
<td>Race, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>56 (49)</td>
<td>41 (64)</td>
<td>.076</td>
</tr>
<tr>
<td>Afro-Caribbean/black</td>
<td>32 (28)</td>
<td>17 (27)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>10 (9)</td>
<td>4 (6)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>16 (14)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Changed partner or had a new partner in previous 3 months, n/N (%)</td>
<td>76/112 (68)</td>
<td>32/64 (50)</td>
<td>.019</td>
</tr>
<tr>
<td>Median no. of partners during previous 3 months (range)b</td>
<td>1 (0–6)</td>
<td>1 (0–7)</td>
<td>.028</td>
</tr>
<tr>
<td>Median no. of partners during lifetime (range)c</td>
<td>15 (1–100)</td>
<td>10 (4–100)</td>
<td>.69</td>
</tr>
<tr>
<td>Infectious agent, n/N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td>41/103 (40)</td>
<td>2/58 (3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>31/110 (28)</td>
<td>4/59 (7)</td>
<td>.001</td>
</tr>
<tr>
<td>Ureaplasmas</td>
<td>33/109 (30)</td>
<td>10/62 (16)</td>
<td>.045</td>
</tr>
</tbody>
</table>

*P* values were determined by use of *t* test for age, by use of Wilcoxon rank sum test for number of partners in the 3 months prior to the study period and for the number of partners during the patient’s lifetime, and by use of χ² test for all other variables.

b Analysis based on 114 men with urethritis and 60 men without urethritis.

c Analysis based on 109 men with urethritis and 37 men without urethritis.

initial presentation, or more frequently if clinically indicated. Five case patients were not invited to do this and were lost to follow-up. At follow-up, symptoms and signs were recorded, an urethral Gram-stained smear was prepared, and an FPU specimen was collected if the patient had had NGU at the previous attendance; otherwise, only an FPU specimen was collected. Investigations on patients who had a recurrence of urethritis were the same as those undertaken at the initial visit. Urethral inflammation was diagnosed at follow-up if there were either ≥5 PMN leukocytes per HPF in ≥5 fields of a Gram-stained urethral smear, or ≥10 PMN leukocytes per HPF in ≥5 fields of a Gram-stained thread from the FPU specimen. The urethral smear was evaluated first; the FPU was examined for pyuria only if the urethral smear tested negative or the results were not available.

Patients with persisting urethritis were treated with enteric-coated erythromycin, 500 mg given 4 times per day for 2 weeks, together with metronidazole, 400 mg given twice per day for 5 days, and again informed that they should abstain from sexual intercourse. If urethritis had resolved, the patient was advised that he could be sexually active again unless his partner or partners had not received treatment, and he was again informed that his partner or partners should be treated before resuming sexual intercourse. Patients who had persistent urethritis after 4 weeks of treatment were asked to reattend 1 week later when an FPU specimen was collected.

Definitions of persistent and recurrent NGU and chronic NGU. Urethritis that persisted up to the first follow-up visit was defined as “persistent.” Urethritis that recurred in patients who did not have persistent urethritis at their first follow-up visit was defined as “recurrent NGU.” Urethritis that occurred as long as a follow-up visit 30–92 days after the commencement of treatment was defined as “chronic NGU.” “Persistent chronic NGU” was defined as urethral inflammation present in patients who had persistent urethritis from presentation to their first follow-up visit after 29 days. “Recurrent chronic NGU” was defined as urethritis that recurred in patients who did not have persistent urethritis at their first follow-up visit after 29 days.

Detection of *C. trachomatis*, *M. genitalium*, and ureaplasmas. Specimens were handled as described elsewhere [5, 13]. The MicroTrak direct fluorescent antibody (DFA) test (Syva) was used to detect *C. trachomatis* elementary bodies in a urethral smear and a deposit from an FPU specimen collected at initial presentation. One or more elementary bodies were regarded as a positive result [5]. At follow-up visits, the urine deposit only was tested in this way on at least 1 occasion. The PCR assay for *C. trachomatis* was undertaken only on specimens that were obtained at initial presentation from patients with
acute NGU who subsequently reattended. This was an in-house that was assay used in a manner that is described elsewhere [18]. The PCR assay was repeated at first follow-up on spec-

imens from patients who were chlamydia-positive at presenta-
tion. It was undertaken again on specimens from any patient who had persistent urethritis after 4 weeks of treatment, the last test being done at the third follow-up visit, when antibiotics would not have been taken for 1 week. The PCR assay was again used to test specimens from patients who were cured but who subsequently relapsed.

In the case of acute NGU, a man was considered to be chlamydia-positive if either the urethral smear or urine deposit was positive when examined by use of the DFA test. A man was considered to be chlamydia-negative only if both smear and urine deposit preparations were available and if DFA test results of which were negative. In the prospective cohort study, a man was considered to be chlamydia-positive during follow-

up if the result of either the urethral smear DFA test or the urine deposit DFA or PCR test result was positive. A man was considered to be chlamydia-negative only if any 2 tests were evaluable and had negative results; otherwise, he was considered unevaluable for C. trachomatis. These tests were undertaken in a blinded fashion, and the results not disclosed until clinical information was available.

PCR assay for M. genitalium [5, 19] was undertaken on spec-

imens from patients at initial presentation, at the next follow-

up visit on specimens from patients who were M. genitali-

um–positive initially, and at the third follow-up visit if urethritis persisted. The PCR assay was undertaken again on specimens from any patient who was cured but who subsequently relapsed.

To take into account findings on retesting that were published in 1993 [5], 4 more M. genitalium–positive men with acute NGU were identified. A man was considered to be M. geni-

talium–positive at presentation and during follow-up if the PCR test result was positive. A man was considered to be M. geni-

talium–negative at presentation if the PCR test result was neg-

ative; otherwise, he was considered unevaluable. During follow-

up, a man was considered to be M. genitalium–negative if the PCR test result was not positive and if urine was available for PCR analysis; otherwise, he was considered unevaluable.

Ureaplasmas were detected by use of a culture method de-

scribed elsewhere [20]. A 1.0-mL aliquot of the FPU specimen obtained before centrifugation was added to a culture medium that contained urea. Specimens were diluted in serial 10-fold steps, and the highest dilution that produced a color change (yellow to pink) after incubation at 37°C was regarded as contain-
ing 1 color-changing unit. The procedure was undertaken on specimens that were collected at presentation and at each visit during follow-up. A patient was considered to be urea-

plasma-positive if ureaplasmas were detected, ureaplasma-neg-

ative if they were not, and unevaluable if no urine specimen was available for analysis.

**Statistical analysis.** Groups were compared by use of ¥2-

Fisher’s exact test, t test, or the Wilcoxon rank sum test where appropriate. Logistic regression was used for multivariate analy-

ses. Results are presented as ORs with 95% CIs. All analyses were performed by use of Stata, version 6.0 (Stata).

**RESULTS**

**NGU.** On univariate analysis, C. trachomatis, M. geni-

talium, and ureaplasmas were associated with acute NGU (table 1). Because there is some evidence that the number of urea-

plasmas per milliliter may be important in the development of NGU, we also undertook a quantitative analysis. On the basis of the results of the Wilcoxon rank sum test, the larger the number of color-changing unit of ureaplasma organisms per milliliter of specimen, the more likely was the patient to have acute urethritis (data not shown).

Multivariate analysis was undertaken to control for potential confounding by age, race, sexual lifestyle, and coinfection. Only C. trachomatis and M. genitalium remained significantly associated with urethritis (OR 13.0, 95% CI, 2.6–64.5; and OR, 17.9, 95% CI 2.0–160, respectively). Similar results were ob-

tained when the variables for which data were incomplete (race and lifetime number of sexual partners) were removed from the model. The analysis was repeated, taking into account the number of color-changing units of ureaplasma organisms per milliliter, but this did not alter the findings.

**Demographic and sexual lifestyle characteristics of the control group of men without NGU in relation to the presence of ureaplasmas.** To investigate why, in multivariate analysis, the detection of ureaplasmas was no longer associated with acute NGU, the demographic and sexual lifestyle associations of urea-

plasmas in men without NGU were evaluated. The data for 62 men were evaluable, and the results are shown in table 2. The detection of ureaplasmas was associated with younger age ($P = .028$), recent change in sexual partner ($P = .038$), and fewer lifetime sexual partners ($P = .022$). Because the data on the lifetime number of sexual partners were incomplete and because there were no ureaplasma-positive patients who were of “other” race (i.e., neither white nor black), these 2 variables were excluded from multivariate analysis. In this model only recent change in sexual partner was significant (OR, 15.1; 95% CI, 1.98–114.5).

**Natural history of NGU after treatment.** Eighty-six (79%) of 109 men with acute NGU attended at least once for follow-

up examination 10–92 days after treatment. The median num-

ber of follow-up visits per patient was 2 (range, 1–5 visits), and there were 200 total follow-up visits. Urethritis was detected during this time, on at least 1 occasion, in 59 (69%) patients;
Table 2. Univariate analysis of demographic and sexual lifestyle associations of ureaplasmas in men without acute nongonococcal urethritis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with ureaplasma (n = 10)</th>
<th>Patients without ureaplasma (n = 52)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean ± SD</td>
<td>25.9 ± 4.5</td>
<td>32.3 ± 8.7</td>
<td>.028</td>
</tr>
<tr>
<td>Race, no. (%)</td>
<td></td>
<td></td>
<td>.60</td>
</tr>
<tr>
<td>White</td>
<td>6 (60)</td>
<td>33 (63)</td>
<td></td>
</tr>
<tr>
<td>Afro-Caribbean/black</td>
<td>4 (40)</td>
<td>13 (25)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
<td>4 (8)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0 (0)</td>
<td>2 (4)</td>
<td></td>
</tr>
<tr>
<td>Changed partner or had a new partner in previous 3 months, no. (%)</td>
<td>8 (80)</td>
<td>23 (44)</td>
<td>.038</td>
</tr>
<tr>
<td>Median no. of partners in previous 3 months (range)</td>
<td>1 (1–2)</td>
<td>1 (0–7)</td>
<td>.73</td>
</tr>
<tr>
<td>Median no. of lifetime partners (range)</td>
<td>8 (5–10)</td>
<td>12 (4–100)</td>
<td>.022</td>
</tr>
</tbody>
</table>

* P values were determined by use of t test for age, by use of Wilcoxon rank sum test for number of partners in the 3 months prior to the study period and for the number of partners during the patient’s lifetime, and by use of χ² test for all other variables.

b Analysis based on 10 men with ureaplasma and 48 men without ureaplasma.

c Analysis based on 8 men with ureaplasma and 27 men without ureaplasma.

of these, 39 (66%) had a positive urethral smear result and 20 (34%) had pyuria. Eighty men reattended between 10–29 days after treatment and 47 between 30–92 days (figure 1). Overall, 13 patients had recurrent urethritis; in 1 patient, it recurred twice. Reported sexual intercourse during the period after treatment to the first follow-up visit was not associated with the detection of urethritis at 10–29 days. Thus, of 48 men with urethritis at 10–29 days, 20 (42%) had engaged in sexual intercourse, compared with 16 (50%) of 32 men without urethritis (P = .46). Men with chronic NGU—that is, urethritis 30–92 days after treatment—were more likely not to have been sexually active during the study period than were men with no urethritis. Therefore, of the 28 men with chronic urethritis, 8 (64%) had engaged in sexual intercourse, compared with 17 (89%) of 19 men without urethritis (P = .052). Finally, men with recurrent urethritis were not more likely to have had sexual intercourse during the study period than were men with persistent urethritis (data not shown).

**Association of ureaplasmas and M. genitalium with urethritis after treatment of acute NGU.** Only 1 patient was chlamydia-positive, by PCR analysis, on one occasion at follow-up (day 14). Ureaplasmas were detected in specimens at 28 follow-up visits from 21 patients. Of these 21 patients, at initial presentation, 8 had been ureaplasma-positive and 11 had been ureaplasma-negative; 2 were unevaluable for ureaplasmas. Detection of ureaplasmas during follow-up was associated with the presence of urethritis at 10–92 days. Thus, of 21 men who had ureaplasmas detected at some time during 10–92 days after treatment, 19 (90%) had urethritis, whereas, of the 60 men who had no ureaplasmas detected, only 37 (62%) had urethritis (P = .014). We also investigated the association between ureaplasmas and urethritis at each study visit. The prevalence of urethritis was 21 (75%) of 28 men when ureaplasmas were detected, compared with 76 (49%) of 154 men when no ureaplasmas were detected, which was again significantly different (P = .012).

**M. genitalium** was detected in specimens at 8 follow-up visits from 7 patients; 1 of these patients was M. genitalium–negative at presentation. Detection of M. genitalium at follow-up was associated with the presence of urethritis, at 10–92 days, although this did not reach statistical significance. Therefore, of 7 men who had M. genitalium detected at some time 10–92 days after treatment, 7 (100%) had urethritis at follow-up, whereas, of the 75 men in whom M. genitalium was not detected, 49 (65%) had urethritis (P = .091). We also investigated the association between M. genitalium and urethritis at each study visit. The prevalence of urethritis was 7 (88%) of 8 when M. genitalium was detected, compared with 90 (52%) of 174 when no M. genitalium was detected (P = .047).

Multivariate analysis was performed to control for the potential association of one microorganism with another. This showed that both ureaplasmas and M. genitalium were associated independently with urethritis during each follow-up visit: OR, 3.39, 95% CI, 1.36–8.46, and OR, 7.91, 95% CI, 0.95–65.9, respectively.

**The association of ureaplasmas, M. genitalium, and C. trachomatis with the development of chronic nongonococcal urethritis 30–92 days after treatment.** Nine men had ureaplasmas detected 30–92 days after treatment. Of these, 5 had...
been sexually active and 4 had not been sexually active before the visit on which ureaplasmas were detected. The detection of ureaplasmas was associated with urethritis 30–92 days after treatment (table 3), although this did not reach statistical significance (P = .12). If only men with chronic NGU who had either symptoms or signs were considered, the detection of ureaplasmas was significantly associated with the development of this condition (P = .042; table 3). In patients who had symptoms or signs but who did not have objective evidence of urethritis, ureaplasmas were not detected significantly more often than they were in patients without either inflammation or symptoms or signs (data not shown).

Two men had *M. genitalium* detected 30–92 days after treatment. One of them had a recurrence of urethritis after sexual intercourse. The other also had a recurrence of urethritis but had not been sexually active. However, he was *M. genitalium*–positive, as determined retrospectively, but urethritis-negative at his first follow-up visit after completion of 14 days of doxycycline treatment, and, therefore, he did not receive further treatment. At a later follow-up visit, on day 33, he had urethritis and was again *M. genitalium*–positive. Both men had symptoms or signs of urethritis. No patients had *C. trachomatis* detected, compared with men with persistent chronic NGU (data not shown).

**DISCUSSION**

The results of this study add further support to the mounting evidence that *M. genitalium* causes urethritis in men. They also provide seemingly paradoxical evidence on ureaplasmas as a cause of urethritis, in that ureaplasmas were not associated with acute NGU but were associated with urethritis after treatment. Also, the detection of either ureaplasmas or *M. genitalium* was associated significantly with the development of chronic NGU with symptoms or signs 30–92 days after treatment, but not with men who had no objective urethritis or symptoms or signs.

We have included in the study patients with either a positive urethral smear result or pyuria, as discussed elsewhere [13]. There is no consensus as to how long urethritis persists or how often it recurs after treatment before it is called chronic. We have chosen 30 days, which we believe is clinically pragmatic, because the completion of at least 1 course of treatment would have occurred by that time. In fact, Hooton et al., when they investigated the etiology and treatment of persistent or recurrent NGU, included only those who had the condition for more than 4 weeks or who had received treatment for this condition within 3 months of enrollment [21, 22]. The situation is further complicated because at follow-up, after treatment for acute NGU, patients can be divided into 4 broad clinical categories: (1) clinically asymptomatic patients (no symptoms or discharge) with no objective evidence of urethral inflammation; (2) clinically symptomatic patients (symptoms, discharge, or
followed up for 3 months, the detection of the presence of ureaplasmas. In addition, when patients were either symptoms or signs of urethritis; of these 15 men, 7 (47%) had recurrent disease.

In this study, 28 (26%) men had chronic NGU, but only 15 (14%) had either symptoms or signs of urethritis; of these 15 men, 7 (47%) had recurrent disease.

*M. genitalium* has now been associated with acute NGU by several groups of investigators, including ourselves [5–10]. Here, we have shown that the association is also independent of the presence of ureaplasmas. In addition, when patients were followed up for 3 months, the detection of *M. genitalium* during this period was associated with the presence of urethritis. Indeed, any person who was PCR-positive for this mycoplasma during the follow-up period had urethritis at the same time.

The detection of ureaplasmas in the lower genital tract of men and women has been associated with the lifetime number of sexual partners [23, 24]. Failure to adequately control for this in case-control studies may be a reason why ureaplasmas have been associated with acute NGU in some studies but not in others. In the present study, in addition to ureaplasmas having been associated with *C. trachomatis* and *M. genitalium*, ureaplasmas were associated with acute NGU on univariate analysis. However, the association was not seen when we controlled for the other microorganisms and for sexual lifestyle in the multivariate analysis. On the other hand, the detection of ureaplasmas during the follow-up period was significantly associated with urethritis, and this association was independent of the detection of either *C. trachomatis* or *M. genitalium*. Previous studies have shown it a difficult organism to eradicate from the genital tract of both men and women [25, 26].

The question then arises, why should ureaplasmas appear not to be associated with acute NGU, but appear be associated with the subsequent development of urethritis after treatment? In this regard, it is perhaps unwise to ignore evidence, reviewed on several occasions [3, 4], that suggests that these organisms may cause acute disease occasionally. We speculate that ureaplasmas do cause acute urethritis in a few patients and that our failure in this study to show that this is so is because of the high rate of asymptomatic carriage of ureaplasmas in the control group of men without urethritis. It is a common microorganism in the female genital tract [24, 25], and we would therefore expect a control group selected from attendees at a department of genitourinary medicine to have a relatively high carriage rate. In fact, we found that the detection of ureaplasmas in the control group was associated with a recent change in sexual partner, which suggests recent acquisition via sexual means. Furthermore, it has been shown in a human volunteer experiment that intra-urethral inoculation of ureaplasmas on the first occasion produced infection without disease, but by the fourth inoculation, there was infection without disease [27]. It seems likely, therefore, that

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ureaplasma-positive</th>
<th>Ureaplasma- or <em>M. genitalium</em>-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive°</td>
</tr>
<tr>
<td>Patients with evidence of urethritis at 30–92 days</td>
<td>8 (88.9)</td>
<td>19 (54.3)</td>
</tr>
<tr>
<td>Patients with no urethritis at 30–92 days</td>
<td>1 (11.1)</td>
<td>16 (45.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>9 (100)</td>
<td>35 (100)</td>
</tr>
<tr>
<td>Patients with urethritis at 30–92 days who had either symptoms or signs</td>
<td>5 (100)</td>
<td>9 (42.9)</td>
</tr>
<tr>
<td>Patients with no urethritis at 30–92 days who had no symptoms or signs</td>
<td>0 (0)</td>
<td>12 (57.1)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5 (100)</td>
<td>21 (100)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients.

° Two men were *M. genitalium*-positive and both had urethritis with either symptoms or signs. No man was chlamydia-positive during this time.

° For patients who tested positive for ureaplasma versus patients who tested negative, *P* = .12. For patients who tested positive for ureaplasma or *M. genitalium* versus patients who tested negative, *P* = .028. *P* values determined by use of Fisher’s exact test.

° For patients who tested positive for ureaplasma versus patients who tested negative, *P* = .042. For patients who tested positive for ureaplasma or *M. genitalium* versus patients who tested negative, *P* = .005. *P* values determined by use of Fisher’s exact test.
ureaplasmas may cause urethritis on initial exposure but less so subsequently, as tolerance develops with time. Therefore, the failure to show an association of ureaplasmas with acute NGU may be because of the high carriage rate in sexually active men who attend departments of genitourinary medicine who have become tolerant to the infection.

The results of this study suggest that both ureaplasmas and M. genitalium are important causes of chronic NGU with symptoms or signs 30–92 days after treatment, but not chronic NGU without symptoms or signs, or symptoms or signs without objective urethritis. This would appear to be analogous to acute NGU, where an infectious cause is associated with symptoms or signs [28, 29]. Why ureaplasmas should persist despite antibiotic therapy and no sexual intercourse is not fully understood. It is possible that some of these ureaplasma strains were resistant to tetracycline, such resistance having been associated before with chronic NGU [12, 14].

The evidence that ureaplasmas and M. genitalium cause urethritis in men raises the issue of whether there should be routine testing for these microorganisms in clinical practice. Before the development of PCR, reliable detection of M. genitalium was not possible, and such technology is still only available within research settings. However, commercial nucleic acid amplification techniques are now readily available for the detection of both C. trachomatis and N. gonorrhoeae in routine clinical samples, and so there is the potential for the development of such commercial technology to detect M. genitalium and ureaplasmas in men with urethritis. NGU has a significant morbidity in men and is associated with an increased risk of HIV transmission [15, 30]. Furthermore, there is some evidence that M. genitalium causes cervicitis [31], and it may be a cause of pelvic inflammatory disease [32], so a clinical picture similar to that induced by C. trachomatis might emerge.

For these reasons, we believe a clinical diagnostic molecular test would be helpful for the detection of M. genitalium in both men and women, although we acknowledge that the majority of cases of acute NGU can be managed clinically without recourse to such a sophisticated test. However, the use of such a test, we believe, may also be of value with regard to management of men with chronic NGU, particularly those with recurrent disease. The situation with ureaplasmas is more complex. Culture is relatively inexpensive, but our results suggest that it may not be particularly sensitive, as evidenced by the number of patients who were ureaplasma-positive at follow-up but ureaplasma-negative at presentation. Whether a molecular approach would improve sensitivity is unknown, and even if it were known, a molecular approach may prove to be unhelpful unless it were quantitative in nature. Furthermore, routine screening would pose problems, because many subjects carry ureaplasmas asymptptomatically and eradication is difficult, particularly in women [25, 26]. Overall, it seems that improved detection is not warranted, at least currently in routine practice, but detection of ureaplasmas may be of value in managing patients with chronic NGU.

These results provide further evidence that M. genitalium and ureaplasmas are causes of urethritis in men and that they are probably a cause of chronic NGU 30–92 days after treatment of acute NGU, particularly when symptoms or signs are present.

Acknowledgments

We thank the medical and nursing staff of the Jefferiss Wing of St. Mary’s Hospital (Paddington, London) for their help and support with this study.

References