MYCOPLASMA GENITALIUM

Mycoplasma genitalium: prevalence, clinical significance, and transmission

C Anagrius, B Loré, J S Jensen

Methods: M genitalium and C trachomatis were detected by polymerase chain reaction from urethral and endocervical swab specimens in a cross sectional study among 445 female and 501 male STD clinic attendees. Partners of 26 female and 26 male M genitalium positive index patients were examined.

Results: The prevalence of C trachomatis and M genitalium was 4% and 6.3%, respectively, among the women and 5.4% and 6%, respectively, among the men. Dual infections were uncommon. M genitalium was strongly associated with urethritis in both men and women and with cervicitis in women. Among M genitalium infected men, symptomatic urethritis was more common than asymptomatic urethritis. M genitalium and C trachomatis were not associated with symptoms of urethritis or cervicitis in women. Of 26 male partners of M genitalium positive female index patients, 38% were positive, and 77% of the negative partners had symptoms of urethritis. The concordance rate for 22 female partners of male index patients was 45%. For both men and women the M genitalium prevalence was significantly higher in partners of M genitalium positive index patients than in M genitalium negative index patients with urethritis and/or cervicitis.

Conclusions: M genitalium is associated with urethritis in both men and women and with cervicitis in women. A high concordance rate was found among sexual partners of M genitalium infected patients, indicating that the infection is sexually transmitted.

Partner notification study

In order to obtain information about the transmission of M genitalium, sexual partners of M genitalium infected patients were traced whenever possible in the same way as partner notification for C trachomatis is performed in Sweden. According to this legislation, sexual partners within at least 6 months should be traced and examined as follows.

1. Patient referral: partners are informed by patient.
2. Provider referral: patient reports name and address or at least telephone number of partner to the contact tracer, a health worker.
3. Conditional approach: initial patient or provider referral is followed by a stronger provider referral after an agreed interval. If a named partner refuses to be examined the medical officer of health is informed.

However, since M genitalium is not included in the legislation, we tried to motivate index patients to ask their partners to be examined. As a minimum, partners in “ongoing relationships” were examined. No partner referral was practised and no letters were sent to partners. M genitalium positive patients identified from November 1997 through December 2001 were included as index patients. During this period, only selected patients were examined for M genitalium. Contact tracing during the prevalence study was not practicable because of a delay between examination and reporting.

Abbreviations: FVU, first void urine; hpf, high power field; NCNGU, non-chlamydial non-gonococcal urethritis; NGU, non-gonococcal urethritis; PCR, polymerase chain reaction; PMNLs, polymorphonuclear leucocytes
the availability of the test result. For comparison, partners of index patients treated for urethritis or cervicitis without known infection with *C. trachomatis*, *Mycoplasma genitalium*, or *N. gonorrhoeae* (non-specific infection) were examined. This was performed during 2003 as data concerning partner notification in non-specific infection were not available earlier.

**Clinical assessment**

Patients were regarded as having symptoms of urethritis if they complained of dysuria or urgency and for males if they had noticed a discharge. In females, discharge was regarded as a symptom of cervicitis.

Microscopic urethritis was diagnosed if >4 polymorphonuclear leukocytes (PMNLs) per high power field (hpf) (1000× magnification) were observed in >4 fields in a methylene blue stained smear of urethral secretion collected with a plastic loop from both men and women.

Microscopic cervicitis was diagnosed if ≥30 PMNLs per hpf were observed in >4 fields in a methylene blue stained smear of cervical secretion collected with a cotton tipped swab.

**Clinical specimens for microbiological analysis**

During the prevalence study, male urethral specimens were taken with a cotton tipped swab transported in a tube with 2 ml 2-SP medium. From women, endocervical and urethral swabs were transported in a tube with 2-SP medium. During the partner notification study, specimens from men consisted of first void urines (FVU) and from females endocervical swabs were placed in the tube containing the woman’s FVU specimen.

The specimens were refrigerated and transported to the laboratory within 1–18 hours. Specimens in 2-SP were frozen before testing, whereas urines were not.

**Microbiological methods**

PCR for *C. trachomatis* was performed by the Roche Cobas Amplicor system (Roche Diagnostics Scandinavia AB, Bromma Sweden) following the guidelines from the manufacturer. PCR tests for *C. trachomatis* and *M. genitalium* were performed on the same specimen.

Sample preparation for the *M. genitalium* PCR was performed by centrifuging 250 μl of the specimen in 2-SP medium at 30 000 × g for 15 minutes, resuspending the pellet in 50 μl lysis buffer (Roche *C. trachomatis* sample preparation kit) with 200 μg protease K/ml, incubating at 55°C for 30 minutes, and at 94°C for 10 minutes. After heat treatment, 50 μl specimen diluent (Roche *C. trachomatis* sample preparation kit) was added. Specimens were left for 30 minutes at room temperature before the PCR was performed. DNA extraction of urine was performed using the automated MagNA Pure LC (Roche Diagnostics) with DNA Isolation Kit I protocol. Before processing in the MagNA Pure, 2 ml of urine was centrifuged for 15 minutes at 30 000 × g. Most of the supernatant was discarded, leaving a final volume of 300 μl. Of this suspension 200 μl was processed and DNA was eluted in a volume of 100 μl. All samples were tested in a PCR using primers for the 16S rRNA gene. Positive results in the first PCR were confirmed in a new PCR using MgPa1 and MgPa3 primers. PCR products were visualised after gel electrophoresis in ethidiumbromide stained gels.

*N. gonorrhoeae* was detected by culture.

**Statistical analysis**

Fisher’s exact test was used for statistic analysis of categorical variables. The Mann-Whitney test was used to test for differences in continuous variables.

**RESULTS**

**Prevalence study**

**Demographics**

Among the 946 examined patients, *M genitalium* was detected in 58 (6.1%); 30 (6.0%) of the 501 men, and 28 (6.3%) of the 445 women. *C. trachomatis* was detected in 45 patients (4.6%); 27 (5.4%) men and 18 (4%) women. Two men and one woman were co-infected with *M. genitalium* and *C. trachomatis*. One man and two women were infected with *N. gonorrhoeae*. They were excluded from the study group, which thus consisted of patients with and without non-gonococcal urethritis and cervicitis.

The median age of the *M. genitalium* infected men was 26 years (range 17–40 years). This was not different from the *C. trachomatis* positive (median 24, range 18–43) or from the *C. trachomatis* and *M. genitalium* negative group (median 26, range 17–67). The *C. trachomatis* positive men, however, were younger than the *C. trachomatis* and *M. genitalium* negative men (p = 0.03) (Mann-Whitney test). The median age of the *M. genitalium* infected women was 25 years (range 19–54 years). This was not different from the *C. trachomatis* positive (median 22, range 18–30) or *C. trachomatis* and *M. genitalium* negative groups (median 25, range 14–55) (Mann-Whitney test).

**Associations between M. genitalium, C. trachomatis, and symptoms and microscopic signs of urethritis in males**

*M. genitalium* was detected in 17 (13.6%) of 125 men with symptomatic urethritis, and in two (1.2%) of 161 men without symptoms or microscopic signs of urethritis (p<0.0001) (table 1). The presence of *M. genitalium* was significantly associated both with symptoms and with microscopic signs. This association was also seen for *C. trachomatis*. If patients co-infected with *M. genitalium* and *C. trachomatis* were excluded, 17 (61%) of the 28 *M. genitalium* positive men had symptoms compared to 15 (60%) of the 25 *C. trachomatis* positive men, however, were from the *C. trachomatis* positive group (median 24, range 18–43) or from the *M. genitalium* and *C. trachomatis* negative group (median 26, range 17–67). The *C. trachomatis* positive men, however, were younger than the *C. trachomatis* and *M. genitalium* negative men (p = 0.03) (Mann-Whitney test). The median age of the infected men was 25 years (range 19–54 years). This was not different from the *C. trachomatis* positive (median 22, range 18–30) or *C. trachomatis* and *M. genitalium* negative groups (median 25, range 14–55) (Mann-Whitney test).

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>M–S– (n = 161)</th>
<th>M–S+ (n = 125)</th>
<th>p Value</th>
<th>M–S– (n = 151)</th>
<th>M–S+ (n = 61)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg+Cl−</td>
<td>2 (1.2%)</td>
<td>17 (13.6%)</td>
<td>&lt;0.0001</td>
<td>9 (6%)</td>
<td>0.03</td>
<td>0 (%)</td>
</tr>
<tr>
<td>Mg−Cl+</td>
<td>0</td>
<td>15 (12.0%)</td>
<td>&lt;0.0001</td>
<td>9 (6%)</td>
<td>0.001</td>
<td>1 (0.2%)</td>
</tr>
</tbody>
</table>

Total number of men in the study was 500. Two men co-infected with *M. genitalium* and *C. trachomatis* were excluded from the comparison. p Values given for comparison with the M–S− group.
Associations between *M* genitalium, *C* trachomatis, and symptoms and microscopic signs of urethritis and cervicitis in females

Of the 443 examined women, 130 had symptoms as well as microscopic signs of urethritis and/or cervicitis. *M* genitalium was detected in 16 (12.3%) of those with and in three (2.2%) of the 138 women without urethritis or cervicitis (p = 0.001). One of the women with microscopic signs was co-infected with *M* genitalium and *C* trachomatis (table 2). Symptoms regardless of microscopic signs were not associated with *M* genitalium infection, whereas microscopic signs of urethritis and/or cervicitis were strongly associated with *M* genitalium infection (table 2). For the *C* trachomatis infected women, no association could be shown between symptoms and microscopic signs (table 2). Symptoms of urethritis or cervicitis were reported by 18 (67%) of the 27 *M* genitalium positive women compared to six (35%) of the 17 *C* trachomatis positive women (NS) (table 2).

Microscopic signs of cervicitis without concomitant urethritis were seen in 30 of the women. Of these, *M* genitalium was detected in four (13.3%) compared to six (2.6%) of the 227 women without microscopic signs (p = 0.02) (table 3A). Microscopic signs of urethritis without concomitant cervicitis were seen in 129 women. Of these, *M* genitalium was detected in 11 (8.5%) compared to six (2.6%) of women without microscopic signs (p = 0.02). *M* genitalium was significantly associated with cervicitis regardless of concomitant urethritis (p = 0.006) (table 3A) and with urethritis regardless of concomitant cervicitis (p = 0.005) (table 3B). *C* trachomatis was not associated with urethritis or cervicitis (table 3).

If dysuria and/or urgency were considered symptoms of urethritis and discharge was considered a symptom of cervicitis, no correlation between the presence of microscopic urethritis or cervicitis and their corresponding symptoms could be found. This held true for both *M* genitalium positive, *C* trachomatis positive and *M* genitalium and *C* trachomatis negative patients. Although the number of patients studied was small, not even a trend was observed towards the expected correlation (table 4).

Partner notification study

Partner notification was performed for 52 index patients, 26 women and 26 men, infected with *M* genitalium.

The 26 *M* genitalium infected women reported 38 male partners. Twenty six (68%) of the male partners were examined for *M* genitalium and 10 (38%) were positive.

Examination was performed on 22 (73%) of 30 female partners reported by 26 male index patients. Ten (45%) were infected.

Symptoms and microscopic signs in partners with negative PCR for *M* genitalium are presented in table 5. Surprisingly, six (50%) of the 13 *M* genitalium negative partners of *M* genitalium positive women, where this information was available, had microscopic urethritis and 10 (77%) had symptoms. Thus, it could be considered likely that at least some of these men were indeed infected with *M* genitalium but remained undetected for unknown reasons. Likewise, nine (82%) of the 11 *M* genitalium negative female partners of *M* genitalium positive men had symptoms and/or microscopic signs of cervicitis/urethritis. In the comparison group 61 female and 80 male partners of index patients with NSU were examined. *M* genitalium was found in one (1.6%) of the females and in four (5%) of the males. *C* trachomatis was found in one (1.6%) of the females. Thus, *M* genitalium was found significantly more often in partners of *M* genitalium positive patients than in partners of patients with non-symptomatic urethritis (p<0.0001 for both male and female partners).

DISCUSSION

This is one of the very few true prevalence studies concerning *M* genitalium, in contrast with the many case-control studies published thus far. In this study, *M* genitalium was found more often than *C* trachomatis in both men and women. Furthermore, the prevalence of *C* trachomatis in male patients with NGU was only 9%. The reason for the low prevalence of *C* trachomatis is not clear. *C* trachomatis infection is notifiable and is included in the Swedish legislation on STIs; therefore, partner tracing can be performed more rigorously than in many other countries. In recent years, however, this has not

Table 2  Symptoms (S) and microscopic signs (M) in women infected with *Mycoplasma genitalium* (Mg) and *Chlamydia trachomatis* (Ct), respectively

<table>
<thead>
<tr>
<th></th>
<th>M—S— (n = 138)</th>
<th>M+S— (n = 129)</th>
<th>p Value</th>
<th>M—S— (n = 85)</th>
<th>p Value</th>
<th>M—S— (n = 90)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg—Ct—</td>
<td>3 (2.2%)</td>
<td>15 (11.6%)</td>
<td>0.003</td>
<td>6 (7.0%)</td>
<td>NS (0.09)</td>
<td>3 (3.3%)</td>
<td>NS (0.68)</td>
</tr>
<tr>
<td>Mg—Ct+</td>
<td>6 (4.3%)</td>
<td>5 (3.9%)</td>
<td>NS (0.99)</td>
<td>5 (5.9%)</td>
<td>NS (0.75)</td>
<td>1 (1.1%)</td>
<td>NS (0.25)</td>
</tr>
</tbody>
</table>

Total number of women in the study was 443. One woman co-infected with *M* genitalium and *C* trachomatis was excluded from the comparison. p Values given for comparison with the M—S— group.

Table 3  Microscopic cervicitis (>30 PMNL/hpf) (A) and microscopic urethritis (>4 PMNL/hpf) (B) in 443 women according to *Mycoplasma genitalium* (Mg) and *Chlamydia trachomatis* (Ct) infection status. One woman co-infected with *M* genitalium and *C* trachomatis was excluded from the comparison.

<table>
<thead>
<tr>
<th></th>
<th>No cervicitis no urethritis (n = 227)</th>
<th>Cervicitis no urethritis (n = 30)</th>
<th>p Value</th>
<th>All cervicitis (n = 84)</th>
<th>p Value</th>
<th>Cervicitis and urethritis (n = 54)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Microscopic cervicitis (&gt;30 PMNL/hpf)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg—Ct—</td>
<td>6 (2.6%)</td>
<td>4 (13.3%)</td>
<td>0.02</td>
<td>9 (10.6%)</td>
<td>0.006</td>
<td>5 (9.1%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Mg—Ct+</td>
<td>7 (3.1%)</td>
<td>1 (3.3%)</td>
<td>NS (0.99)</td>
<td>5 (5.9%)</td>
<td>NS (0.31)</td>
<td>4 (7.3%)</td>
<td>NS (0.23)</td>
</tr>
</tbody>
</table>

| (B) Microscopic urethritis (>4 PMNL/hpf) |         |                                 |         |                         |         |                                 |         |
| Mg—Ct—       | 6 (2.6%)                              | 11 (8.5%)                       | 0.02    | 17 (9.2%)               | 0.005   | 5 (9.1%)                        | 0.04    |
| Mg—Ct+       | 7 (3.1%)                              | 5 (3.9%)                        | NS (0.76)| 9 (4.9%)               | NS (0.44)| 4 (7.3%)                        | NS (0.23)|

p Values given for comparison with the no urethritis and no cervicitis group.
been sufficient to ensure a decreasing prevalence of *C. trachomatis* infection in Sweden in general. The age distribution of patients infected with the two pathogens was not significantly different, indicating that they may share a common behavioural and biological profile. The strong association between *M. genitalium* and male urethritis independently of *C. trachomatis* found in this study strongly indicates that it has an aetiological role in urethritis. Our findings confirm results from several other studies regarding the association between *M. genitalium* and NGU in general and NCNGU in particular (see Jensen for review). We found that almost all *M. genitalium* and *C. trachomatis* positive male patients had urethritis (93% and 96%, respectively), whereas only less than two thirds had symptoms (61% and 64%, respectively). For both pathogens, the high proportion of asymptomatic carriers among the infected would facilitate the spread of the infection. Even though many *M. genitalium* patients are asymptomatic, more patients had symptomatic than asymptomatic urethritis in this study. This is in accordance with earlier studies on smaller numbers of patients. 

The remarkably few patients with gonorrhoea reflected the low prevalence of this infection in Sweden. Only 210 cases were reported in 1996 in a population of 8.5 million.

The few studies performed in women have indicated that *M. genitalium* might be a pathogen in cervicitis. This is in agreement with our findings. We detected *M. genitalium* five times more often in women with cervicitis than in those without (13.3% compared to 2.6%). Casin et al., however, did not show such an association. As a definition of cervicitis ≥10 PMNL/hpf was used compared with ≥30 in the present study. They also found a remarkably high prevalence of *M. genitalium* (38%) in comparison with the prevalence of *C. trachomatis* (8%) raising concern about the specificity of the PCR method used.

In the present study, *M. genitalium* was detected three times more often in women with urethritis than in those without, (9.2% compared to 2.6%). This is to our knowledge the first study to show an association between *M. genitalium* infection and female urethritis.

Discharge as reported by the women may be caused by urethritis as well as by cervicitis. Discharge from the urethra is probably a less important symptom in females than in males. Without microscopy, discharge from the vagina caused by bacterial vaginosis or candidiasis cannot be differentiated from discharge caused by cervicitis, and more than one condition may be present at the same time. Bacterial vaginosis may be a marker of STI as it is found more often in women with *C. trachomatis* infection.

The results of a few studies, apart from the present one, have indicated that *M. genitalium* is a STI. Keane et al. studied female partners of men with and without NGU and found *M. genitalium* only in partners of men with NGU. Seven (58%) of the 12 partners of *M. genitalium* positive male index were positive, and similarly, Falk et al. found 67% positive female partners. These figures are slightly higher than the 45% positive female partners of *M. genitalium* positive men found in this study, but not statistically significantly different. The surprising finding that 50% of 13 *M. genitalium* negative male partners of *M. genitalium* positive women had microscopic urethritis and that 77% had symptoms raises concern that the diagnostic methods may need improvement. The recent finding that 20% of urogenital swab specimens contain <1 genome copy/μl of the pretreated specimen indicates that sample preparation methods may need to be improved. Furthermore, it would be interesting to examine first void morning urine specimens from such discrepant couples since this specimen type may provide a higher sensitivity.

A study based on serological data found a significantly higher prevalence of antibodies against *M. genitalium* in STD patients.

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### Table 4: Distribution of symptoms in 443 women according to *Mycoplasma genitalium* (Mg) and *Chlamydia trachomatis* (Ct) infection status and their relation to microscopic cervicitis (≥30 PMNL/hpf) and/or urethritis (≥4 PMNL/hpf). One woman co-infected with *M. genitalium* and *C. trachomatis* was excluded from the comparison.

<table>
<thead>
<tr>
<th>Mg– Ct–</th>
<th>Mg– Ct+</th>
<th>Mg+ Ct–</th>
<th>Mg+ Ct+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervicitis (n=4)</td>
<td>0</td>
<td>2 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>Urethritis (n=11)</td>
<td>6 (55%)</td>
<td>1 (9%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Both (n=5)</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>None (n=6)</td>
<td>1 (17%)</td>
<td>2 (33%)</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 5: Symptoms and signs among 12 Mg– and 10 Mg+ female partners of 26 male index patients and in 16 Mg– and 10 Mg+ male partners of 26 female index patients.**

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mg–</th>
<th>Mg+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Mg–</td>
<td>6* 2 1 2 1</td>
<td></td>
</tr>
<tr>
<td>Female Mg+</td>
<td>4 5 1 0</td>
<td></td>
</tr>
<tr>
<td>Male Mg+</td>
<td>6† 0 4 3 3</td>
<td></td>
</tr>
<tr>
<td>Female Mg+</td>
<td>3 5 0 1 1</td>
<td></td>
</tr>
</tbody>
</table>

*Two also had a history of irregular bleeding.
†One male partner was co-infected with *Chlamydia trachomatis*, and one male partner had arthritis.
M, microscopic urethritis and or cervicitis; S, subjective symptoms of urethritis and or cervicitis. One woman and four men did not have microscopic examination performed.
Mycoplasma genitalium is strongly associated with symptomatic urethritis in both men and women and with microscopic cervicitis in women
Symptomatic urethritis is more common than asymptomatic urethritis in M genitalium positive men
In women, symptoms of urethritis and cervicitis do not correlate with microscopic urethritis and cervicitis
Partners of M genitalium infected men and women were significantly more often infected with M genitalium than partners of patients with non-specific infection. This supports the notion that M genitalium is sexually transmitted

Clinic attendees than in healthy blood donors, indirectly supporting the notion that it is spread via sexual transmission.

In our prevalence study, three men infected with M genitalium had arthritis. In the partner notification study, one man infected with M genitalium and one male partner with negative PCR for M genitalium, but with symptoms and microscopic signs of urethritis also had arthritis. A possible causal association between M genitalium infection and arthritis has been implicated earlier by the detection of M genitalium from the knee joint of a patient with Reiter’s syndrome but this relation needs to be investigated further.

In conclusion, this study has further substantiated the role of M genitalium in lower genital tract infections and documented that M genitalium is sexually transmitted with a transmission rate comparable to that of C trachomatis. Further work is still needed, particularly in establishing the optimal treatment and in documenting upper genital tract disease.

CONTRIBUTORS
CA initiated the study, examined all patients, collected the data, and wrote the first draft of the manuscript; BL was responsible for the N gonorrhoeae, M genitalium, and C trachomatis tests; she provided major contributions to the design of the study, analysis of the data, and writing of the manuscript; JSJ provided advice on the M genitalium tests and the design of the study; he provided major contributions in the data analysis and in writing the manuscript.

References
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