Low prevalence of Mycoplasma genitalium in patients examined for Chlamydia trachomatis

Summary

Background. There is increasing interest in Mycoplasma genitalium as a sexually transmissible pathogen. The clinical picture resembles that of Chlamydia trachomatis infection, but the natural course has not been well defined. There are no national guidelines regarding who should be examined for M. genitalium. Most of the prevalence studies have been carried out in patients attending clinics for sexually transmissible diseases. We have examined the prevalence in samples sent from general practice questioning the diagnosis C. trachomatis.

Material and method. During the period 1 October to 31 December 2010, all the samples sent to Molde Hospital that queried C. trachomatis were examined. Both agents were examined using real time PCR. The PCR for C. trachomatis was performed using a CE labelled and IVD approved method from Roche. The PCR for M. genitalium was performed using an in-house method where the target gene is GAP.

Result. A total of 950 patients were examined (Men n=225, women n=725). The prevalences of M. genitalium and C. trachomatis were 2.0 % and 10.0 % respectively (men 4.0 % and 15.1 %, women 1.4 % and 8.4 %).

Conclusion. Because of the low prevalence, we recommend selection of patients for examination for M. genitalium. The difference in prevalence between the sexes can reflect different indications for sample taking.

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Mycoplasma genitalium is receiving increasing attention as an agent for sexually transmissible disease. The prevalence studies have mainly been carried out in patients attending clinics for sexually transmissible diseases, where the patients constitute a high risk group. By evaluating the prevalence of M. genitalium in samples from general practice, we hoped to find out whether this microbe should be tested for under the same indication as that for Chlamydia trachomatis.

Mycoplasma species are amongst the smallest known bacteria. They lack a cell wall and are therefore not affected by beta-lactams or other cell wall antibiotics. M. genitalium is an extracellular bacterium that utilises the biosynthesis apparatus of epithelial cells (1). Several partner studies confirm that the microbe is sexually transmissible (2). The prevalence has varied in different studies between 0.7 % in an English study of pregnant women and 38 % in a French study of women with abnormal discharge (3, 4).

The clinical picture resembles infection with genital Chlamydia. Urethritis in men is well documented (2). M. genitalium has been found in prostate tissue and semen in patients with prostatitis, but the relevance of this is poorly investigated (2, 5, 6). There is also a possible connection with epididymitis (2). It is not known whether men can develop sequelae from untreated infection.

Investigations on the connection between M. genitalium and cervicitis/urethritis in women have not given clear results. One weakness is that different definitions of urethritis/cervicitis have been used in different studies (2, 7). There are a limited number of studies in which the connection with upper genital infection has been investigated. There seems to be a connection, though the association is probably weaker than with C. trachomatis (8, 9). A Swedish study shows that there is an increased risk of salpingitis after surgical abortion when M. genitalium is found in the lower genital tract (8). The connection with tubular infertility is poorly evaluated, and there are conflicting findings in the two studies that have examined this (10, 11). In two of eight studies, a connection has been shown between M. genitalium in the lower genital tract and spontaneous abortion/premature birth (2).

Material and method

During the period 1 October -31 December 2010, our laboratory analysed for M. genitalium in all the samples sent querying the diagnosis C. trachomatis. The samples were from symptomatic and asymptomatic patients in a broad age range. There was scanty or absent clinical information in the referral notes. Pregnant women were excluded because there was not sufficiently good documentation for giving advice when the results were positive. Those referring were informed in advance by letter. Publication of the material has been approved by the Regional Ethics Committee in Mid Norway and reported to the hospitals safety officer.

The urine samples were collected in sterile polypropylene plastic tubes. Cervical and urethral samples were taken with STD Swab Collection and Transport Set (Roche Molecular Systems Inc.). The urine samples were extracted at MagNA Pure LC, kit Total Nucleic Acid (Roche). The brush samples were extracted by adding Diluent (A-CT/NG Spec Prep, cat.no 20 759 414 122 Roche). The analyses for both agents were real time PCR methods, with TaqMan probes.

The PCR examinations for C. trachomatis were carried out with COBAS TaqMan 48 using a CE labelled and IVD approved met-

Main message

- There is a low prevalence of M. genitalium in patients investigated for C. trachomatis
- Lack of gold standard in the diagnostic procedure for M. genitalium leads to uncertainty about the test's sensitivity and specificity
- Diagnostic procedures for M. genitalium should therefore be confined to patients with symptoms, or findings/demonstrated infection in partner.

hod from Roche. The PCR examinations for M. genitalium were carried out using LightCycler 480 with an internal method validated at our laboratory in autumn 2010. The target gene for the method is GAP, glyceraldehyde-3-phosphate dehydrogenase. Others have found that the detection limit for the method is less than five copies, and no cross reactivity for other species of Mycoplasma has been found (12). 497 urine samples, 35 urethral brushes and 462 cervical brushes from a total of 950 patients were examined (725 women, average age 26 years, SD 9.5 years/225 men, average age 29 years, SD 11.2 years. Seven patients were excluded because of inconclusive results of the PCR examination.

The results were collected from the laboratory's data system, and processed by SPSS, version 17.0. The difference in prevalence between the sexes was estimated using a chi-square test. The age difference between those infected with M. genitalium and those with C. trachomatis was estimated using Mann-Whitney's test.

Table 1 Prevalence of M. genitalium and C. trachomatis in a test population. Number of positive tests (percentage in brackets) is given separately for each sex. Age distribution in patients with positive results is given with the average age and standard deviation (SD)

	Women (n=725)	Men (n=225)	Total (n=950)
Chlamydia trachomatis – number (%)	61 (8.4)	34 (15.1)	95 (10.0)
Mycoplasma genitalium – number (%)	10 (1.4)	9 (4.0)	19 (2.0)
Average age number – (SD)	26.0 (9.5)	28.8 (11.2)	26.8 (10.0)
Average age positive C. trachomatis year – (SD)	21.8 (5.9)	25.0 (5.3)	23.0 r (0.6)
Average age positive M. genitalium year – (SD)	24.6 (4.8)	32.4 (12.4)	28.3 (2.2)

Results

The total prevalence in the 950 patients was 2.0 % (n=19) for M. genitalium and 10.0 % (n=95) for C. trachomatis. The prevalences in men were 4.0 % and 15.1 % respectively, and in women they were 1.4 % and 8.4 %. Two women (0.3 %) and two men (0.9 %) had a co-infection. The difference in prevalences between the sexes was significant, with p=0.025 for M. genitalium and p=0.005 for C. trachomatis. The average age of M. genitalium positive patients was 28.3 years (SD 2.2 years), compared with 23.0 years (SD 0.6 years) for C. trachomatis. The age difference was significant, p=0.001 (Tab. 1).

Discussion

The strength of our material was that it was in fact the selection that would have been examined if the general practitioner had ordered diagnosis of M. genitalium based on the same criteria as those for C. trachomatis. The prevalence in this sample, seen in relation to the severity of the disease, gives an indication of whether or not this practice should be introduced. Possible geographic differences in prevalence should be taken into account.

Diagnostic procedures for M. genitalium are based on gene technology. So far, there are no commercial tests on the market, but several in-house methods (in-house) have been published (13). The detection limit of our method is under five copies, and it has not been possible to demonstrate cross reactivity with other mycoplasma species (14).

Validation of the method indicates specificity of 100 % or just below this. Unfortunately there is no gold standard and definitive sensitivity and specificity are not known. With the low prevalence of M. genitalium in our sample, there is a risk of a high proportion of false positive test results even with a small deviation from 100 % specificity. A specificity of 98 % would be sufficient for concluding that all the positive samples in our total sample were false positive. Increasing the pre-test probability in the sample is therefore important potentially to increase the positive predictive value.

By comparing urine samples, brush samples from the urethra, cervix, and vaginal wall from the same patient, it has been shown that the examination for M. genitalium is relatively less sensitive in some of the sample material than that found with C. trachomatis (14, 15). Lillis et al. give the relative sensitivity for M. genitalium in women as 61.4 % for urine, 74.3 % for cervical brush, and 85.7 % for vaginal brush. A combination of cervical brush and vaginal brush gave a sensitivity of 95.7 % (16). They recommend taking a sample from the cervix and then drawing the same brush along the vaginal wall. The urine samples in this study had been frozen before testing/re-testing. Others have shown that the sensitivity decreases when the urine is frozen and that urine is more sensitive than a cervical brush, 88 % and 71 % respectively (15, 17).

As regards the results in men, one study showed a relative sensitivity of 97.6 % for urine and 82.5 % for a urethra sample (15). There were no vaginal brushes in our study, and only 44 of the patients had samples from more than one locality. Given a specificity for our test of 100 %, the prevalence of M. genitalium is underestimated in our findings. An estimate where the relative sensitivity described by Lillis et al. for women and a sensitivity of 95 % and 80 % for urine and urethra brush from men gives a total prevalence of 2.5 % in our sample. In women the prevalence becomes 1.9 % and in men it is 4.4 %. (The 44 patients where samples were taken from two localities are not included in the estimate).

The difference in prevalence between the sexes can reflect different indications for sample taking. Asymptomatic women are probably often screened in connection with a gynaecological examination for other reasons. Men probably consult doctors because of symptoms, own-recognised risk behaviour or as part of tracing an infection. However, it is known that symptoms compatible with urethritis are more specific of infection in men than of infection in women (18, 19). A difference in prevalence between the sexes is therefore expected, even when the selection for sample taking is based on symptoms.

By neglecting to screen for M. genitalium there is a risk of creating a persistent asymptomatic reservoir. This reservoir is small in our sample, where there were no purposeful attempts to diagnose or treat. This could be because M. genitalium will generally be eradicated in the treatment of suspected infectious urethritis, assuming that this is treated with azithromycin and not doxycycline (20). The fact that the reservoir is smaller for C. trachomatis may have several explanations. One study shows that M. genitalium more often gives symptoms than C. trachomatis (21). This indicates that a higher proportion of M. genitalium infected patients consult a doctor and are treated. The frequency and time aspect of spontaneous cure of M. genitalium infection has not been clarified. A more rapid spontaneous recovery would also contribute to a smaller reservoir.

The long-term consequence of untreated M. genitalium infection has not been well clarified, but our data indicate that the prevalence is low. The diagnosis of M. genitalium is based on an expensive gene technique analysis: in our opinion the diagnostic evaluation should therefore be confined to patients with symptoms or findings compatible with infection – and to asymptomatic patients when the partner has developed infection. This would also increase the pre-test probability and possibly the predicative value of the test. Examination of asymptomatic patients should also be considered before surgical abortion (2, 8).

According to new guidelines, azithromycin and not doxycycline should be the first choice in the treatment of urethritis. Doxycycline is not expected to affect M. genitalium infection, with the result that it is a problem that the diagnosis of M. genitalium is only available in certain laboratories.

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