

Prevalence of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis* in patients with uncomplicated recurrent urinary tract infections

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Abstract

Aim: To assess the prevalence of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Chlamydia trachomatis* in patients with chronic urinary tract infections (UTIs) and its correlation with leukocyturia and symptoms.

Methods: The study included 220 patients (130 women and 90 men) presenting with chronic voiding symptoms and sterile leukocyturia. Urine, urethral swabs and cervical swabs (for women patients) were taken to determine the presence of these pathogens. Patients were treated by tetracycline and followed up three and six months after initial therapy.

Results: In 186 (85%) out of 220 patients, *U. urealyticum* was found, while *C. trachomatis* was present in 34 patients (15%). In majority of female patients (112 out of 130; 86%) *U. urealyticum* was found. In addition to ureaplasma, in eight patients *M. hominis* was found. *C. trachomatis* was identified in 18 female patients (14%). In 74 out of 90 (82%) male patients *U. urealyticum* was detected while in six of them *M. hominis* was also found. *C. trachomatis* was identified in 16 male patients (18%). *U. urealyticum* was significantly related to leukocyturia, as opposed to *C. trachomatis* ($p < 0,001$). Women had more frequent symptomatology ($p = 0,015$) and higher leukocyturia ($p < 0,001$).

Conclusion: Leukocyturia is more common find in *U. Urealyticum* infection than in patients with *C. trachomatis* infection. It is therefore advisable to test patients with uncomplicated recurrent UTIs for the presence of these atypical pathogens. Symptomatic patients, as well as their partners, should be treated accordingly.

Introduction

Mycoplasma hominis (*M. hominis*) and *Ureaplasma urealyticum* (*U. urealyticum*) and *Ureaplasma parvum* are species in the family *Mycoplasmataceae*, encompassing the smallest bacteria replicating in culture medium. These tiny microorganisms can be found commensal in lower genitourinary tracts of sexually active men and women. Moreover, they cause many disorders such as non-gonococcal urethritis (NGU), postpartum fever, infertility, and pelvic inflammatory disease [1,2]. Their occurrence in the context of urogynaecological diseases has been demonstrated not only in urethritis and cystitis but also in upper renal tract infections, while their role in hyperactive bladder and interstitial cystitis/painful bladder syndrome remains controversial [3].

Chlamydia trachomatis is an obligate intracellular bacterium. During its unique developmental cycle, two different forms are observed, elementary bodies (EBs), which are infectious but not able to divide, and reticulate bodies (RBs), which are metabolically active and able to multiply [4]. *C. trachomatis* is the most common bacterium causing sexually transmitted infections. Most of these infections are asymptomatic and, if not treated, can lead to severe complications, mainly in young women [5].

All of the above-mentioned bacteria are associated with various diseases of the urogenital tract, but they are usually not detected by

routine microbiological diagnosis (negative urine cultures can be seen). Urine analysis usually helps in diagnosis. Sterile pyuria is classified as the presence of more than 5–8 leukocytes per high-power field on microscopy, in the setting of negative urine cultures [6,7]. According to previous studies urogenital mycoplasmas and *C. trachomatis* are frequently found in patients with sterile pyuria [8]. The clinical presentations for sexually transmitted infections (STIs) and urinary tract infections (UTIs) may overlap, and symptoms of dysuria and urinary frequency/urgency occur with both STIs and UTIs [9,10]. There are scarce literature data regarding the presence of urogenital mycoplasmas and chlamydia in relation to persistent leukocyturia and chronic symptoms. Therefore, we decided to detect the presence of *Ureaplasma urealyticum*, *Mycoplasma hominis* and *C. trachomatis* in patients experiencing chronic urinary symptoms and their correlation with leukocyturia.

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Materials and methods

Patients

The research was carried out from December 2013 to December 2017. In this period 580 patients were diagnosed with UTI. The study included patients (male and female) older than 18 years of age who had at least three uncomplicated UTIs in the previous 12 months (cystitis or urethritis) and who experienced persistent voiding difficulties despite treatment and/or leukocyturia (defined as the presence of more than 5 leukocytes per high power field on microscopy). Patients who reported antibiotic use in the previous month, with febrile condition, bacteriuria, positive urine culture, complicated UTIs, kidney diseases, neurogenic bladder, on immunosuppression, diabetes mellitus and pregnancy were excluded from the study.

In our study, we only tested patients with symptomatic uroinfection while we treated their partners without testing since it is a sexually transmitted agent. We explained in detail to each patient the importance of taking therapy for both partners regardless of the fact that the other partner had no symptoms.

Were there more partners? It is difficult to answer such a question because the population of the examined patients were not just young people, and we did not include such questions in the research. However, in conversation with the patients we would inquire about that issue as well.

Patients who were included in the study underwent following tests: detailed anamnesis, ultrasound of urotract, urine analysis and microbiological tests. Clinical parameters included symptoms: discomfort when urinating; urinary frequency (polaisuria) and nocturia, feeling of incomplete voiding, dysuria and suprapubic pain.

Urine samples, urethral swabs and cervical swabs (in women) were analysed. Microscopic examination, urine culture and detection of *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Chlamydia trachomatis* was done on all samples initially and after three and six months. The first line therapy drug was tetracycline while the second line of therapy for the patients with positive tests at check-up visit was levofloxacin.

Urine analysis and culture

A midstream specimen of the first morning urine (clean-catch specimen) was collected for urine analysis and culture test. Only patients with sterile urine culture were included.

For the microscopic examination, a fresh sample of the first morning urine (clean-catch, midstream urine) 10ml was taken, which was centrifuged for 5 min. at 2000 rpm. After centrifugation, the supernatant is discarded, and a volume of 0.2 to 0.5 ml is left inside the tube. The sediment is resuspended in the remaining supernatant by manually shaking the tube. A drop of resuspended sediment is poured onto a glass slide and covered with a slip. The urine sediment specimen is first viewed with 10 x magnification and then 40 x magnification in at least 10 visual fields, and the results were expressed as an average number of cells.

It is considered that in the urine of a healthy person there can be up to five leukocytes per visual field. More than five leukocytes per visual field is considered to be leukocyturia. The presence of ≥ 10 white blood cells/high-power field is indicative of pyuria [11]. As for erythrocytes, normal value was considered the number of up to three E in visual field.

Detection of urogenital mycoplasmas and *C. trachomatis*

The first voided morning specimen was taken in order to prove the presence of *U. urealyticum*, *M. hominis* and *C. trachomatis* respectively. In symptomatic patients without leukocyturia, urethral swab and cervical swab in women were also analysed. For urethral swabs, patients were not allowed to urinate at least three hours before sample taking. The presence of urogenital mycoplasmas, i.e. *Ureaplasma urealyticum/parvum* and *Mycoplasma hominis* from urogenital specimens was determined using commercially available MycoView kit (Zeakon diagnostics, France). The kit enables identification, quantification and susceptibility testing to five antibiotics: levofloxacin, moxifloxacin, erythromycin, clindamycin and tetracycline. We considered positive finding $>10^4$.

Unfortunately, in our regional medical institutions PCR identification of *M. hominis* and *U. urealyticum* is not performed yet. *Chlamydia trachomatis* was detected by real time PCR using Cobas 4800 CT/NG test (Roche) after automatic extraction (Cobas x 480, Roche Diagnostics) and amplification (Cobas z 480, Roche Diagnostics).

Data entered in excel table were processed in IBM SPSS Statistics 20. In data processing we used χ^2 test, Mann Whitney U test. Results were interpreted at a significance level $p < 0.05$.

Statistical analysis included data referring only to the first patients visit.

Results

The research included 220 patients (130 women and 90 men) who met the inclusion criteria. In 186 out of 220 patients (85%) *U. urealyticum* was found while *C. trachomatis* was present in 34 patients (15%). Hundred and twelve out of 130 (86%) female patients had *U. urealyticum* while in eight patients *M. hominis* was also detected. *C. trachomatis* was found in 18 female patients (14%). In 74 out of 90 male patients (82%) *U. urealyticum* was found; while in six of them *M. hominis* was also detected. *C. trachomatis* was detected in 16 male patients (18%).

Mean age of patients included in the study and the number of leukocytes and erythrocytes in urine is presented in Table 1.

Women patients included in the study were significantly younger than men. Leukocyturia in female patients were significantly higher than in men while the erythrocytes count was significantly lower. Leukocyturia differed significantly regarding the pathogen found. Patients with cultures positive to *Ureaplasma urealyticum* had significantly higher leukocyte counts than patients with *Chlamydia trachomatis* detected, as seen in Table 2. Median of leukocytes in urine of patients with positive *U. urealyticum* was for 11 leukocytes (95% CI: 7-15 leukocyte) higher than in patients with *C. trachomatis* ($z=4.4$; $r=0.31$; $p < 0.001$) (r =effect size).

We did not prove statistically significant difference in erythrocyte number between patients with *U. urealyticum* and *C. trachomatis*. In Table 3. there is a correlation of symptoms in relation to gender.

Distribution of patients according to symptoms in relation to gender was statistically significantly different ($\chi^2=62.7$; $p < 0.001$). Strength of association is moderate (Cramers $V=0.543$; $p < 0.001$).

The proportion of only one symptom in the form of discomfort during urinating in men was nine times higher than the proportion of the same problem in women. The proportion of female patients with

Table 1. Presentation of research variables of all patients in relation to gender

Variables		All patients (n=220)	Men (n=90)	Women (n=130)	P
Age (years)	Median (Q1-Q3; min-max)	40 (27-55; 18-87)	48.5 (26-63; 18-87)	39.4 (25-51; 18-83)	<0.001*
Leukocytes in urine	Median (Q1-Q3; min-max)	10 (4-30; 0-40)	8 (4-30; 1-40)	12 (3.5-32.5; 0-40)	0.015*
Erythrocytes in urine	Median (Q1-Q3; min-max)	4 (1-7; 0-40)	4.5 (2-10; 0-40)	3 (0-7; 0-40)	0.007*

*Mann-Whitney U test

Table 2. Leukocyturia in relation to the detected pathogen

		All (n=220)	<i>U. urealyticum</i> Positive (n=186)	<i>C. trachomatis</i> Positive (n=34)	P*
Leukocytes in urine	Median (Q1-Q3; min-max)	10 (4-30; 0-40)	14 (4.8-30; 0-40)	3 (2-6; 1-40)	<0.001*
Erythrocytes in urine	Median (Q1-Q3; min-max)	4 (1-7; 0-40)	4 (0-8; 0-40)	3 (2-5; 1-40)	0.901*

*Mann-Whitney U test

Table 3. Chronic symptoms in male and female patients with uncomplicated urinary tract infections

Symptoms	All	n (%)		p*
		Men	Women	
Discomfort when urinating	44 (20.7)	38 (43.7)	6 (4.8)	<0.001
Discomfort when urinating, frequency/nocturia	34 (16.0)	6 (6.9)	28 (22.2)	
Discomfort when urinating, incomplete voiding	44 (20.7)	5 (5.7)	39 (31.0)	
Discomfort when urinating, frequency, dysuria	23 (10.8)	7 (8)	16 (12.7)	
Other	68 (31.9)	31 (35.6)	37 (29.4)	

*χ² test

problems in the form of discomfort during urinating and frequent urinating with nocturia (two symptoms) was three times higher in women than in men. The proportion of problems in the form of discomfort during urinating and a feeling of incomplete voiding of the bladder (two symptoms) was 5.4 times higher in women than in men. The proportion of three symptoms was almost two times higher in women than in men.

After isolating the agent, therapy was included for both partners. Three months after initial therapy, 22 patients (10%) needed repeated therapy, while 198 (90%) patients had normal results and they did not have any symptoms. At the final check-up, after six months three patients again had *Ureaplasma urealyticum*. Other patients had normal microbiological test results, urine sediment was normal, without leukocyturia, and patients did not complain about any of the previously mentioned symptoms.

Discussion

In persistent or recurrent UTI in adults it is important to distinguish complicated from uncomplicated forms. Complicated UTI implies infections of urinary tract which is anatomically or functionally altered (urodynamics or voiding are abnormal). Uncomplicated UTI occur mainly in otherwise healthy patients with structurally normal urinary tract and intact voiding mechanisms [11]. Our research involved patients with uncomplicated UTI. Due to the shortness of female urethra, women are more prone to urinary tract infections as well as spreading of infection from the genital tract. Approximately 50–60% of women report at least one UTI in their lifetime, and one in three will have at least one symptomatic UTI necessitating antibiotic treatment by age 24 [12]. As in other studies, in our study, women were also more presented with chronic urinary symptoms. Also, in women, leukocyturia was more pronounced than in men.

While analysing leukocyturia, we found that the number of leukocytes was significantly higher in the group of patients with *U. urealyticum* than in the group with *C. trachomatis*.

A lot is already known about mycoplasma-associated genital tract infections, however there is still a lack of clarity on the influence and pathogenic potential of mycoplasma and ureaplasma species in the context of urinary tract infections and other urogynaecological symptoms such as irritable bladder and interstitial cystitis/bladder pain syndrome [3].

The role of normal vaginal flora in the defence against genital colonization with potentially pathogenic adhering *E. coli* has been demonstrated in several studies [13]. In women, disturbed vaginal microflora in fact enables colonization of uropathogens which are part of normal intestinal flora and this can lead to recurring bacterial UTI. However, even after treated bacterial infections, some symptoms of UTI and leukocyturia often persist. In such situations atypical uropathogens should be taken into consideration.

'Symptomatic abacteriuria', i.e. bacterial infection with low counts of uropathogens may present also as the so-called urethral syndrome and several pathogens are implicated such as *Chlamydia*, *Mycoplasma*, *Trichomonas*, *Gonococci*, *Candida* or *Mycobacteria* [11].

Stamm, *et al.* associated the urethral syndrome, that is dysuria and/or frequency without significant bacteriuria with *Chlamydia trachomatis* infection [14] while Matthews' found the same pathogen as cause of sterile pyuria [15].

In our research the frequency of *C. trachomatis* infection in symptomatic women was lower in relation to the proportion of *U. urealyticum* infection. Among 130 women, *C. trachomatis* was identified in 18 patients (14%) while 112 (86%) had *U. urealyticum*. Also, in men,

C. trachomatis was less frequent. As opposed to Matthews research, we have not proved leukocyturia in patients with chlamydia.

Our findings are in agreement with previous studies (Pott's, Baka, Schlicht) proving the fact that although often overlooked or improperly treated, *U. urealyticum* and *M. hominis* infections may account for a large proportion of unexplained chronic voiding symptoms [16-18]. In our research *U. urealyticum* was substantially more prevalent in both genders. Of 220 patients, 186 (85%) had *U. urealyticum* while *C. trachomatis* was present in 34 patients (15%).

In our research, women had a larger number of symptoms in relation to men. In women, discomfort during urination and frequent urination occurred three times more than in men, while combined discomfort during urinating and the feeling of insufficient voiding of bladder occurred five times more in women than in men ($p < 0,001$). In male patients the dominant symptom was discomfort during urination ($p < 0,001$).

Our patients were given tetracycline (doxycycline twice daily) 14 days for *M. hominis* and *U. urealyticum* infection and 10 days for *C. trachomatis* infection. On the first check up after three months, repeated therapy was given to the total of 22 patients (10%). After six months, three patients were again positive to *U. urealyticum*. Other patients had normal microbiological test results, urine sediment was normal, and patients felt no symptoms.

A question is raised when to test the patients after completed therapy and for how long to follow them. Do we often get a falsely negative result if we take samples too early? It often happens that both partners are not treated.

According to our experience, we think that sample testing should be carried out after three months from the therapy, while earlier testing should be carried out only in patients who experience repeated symptoms. We underline the need to treat both partners.

Conclusion

In patients with frequent and repeated uroinfections it is advisable to assess the presence of *U. urealyticum*, *M. hominis* and *C. trachomatis*. Persisting leukocyturia is more often found in *U. urealyticum* infection, while normal urine sediment in symptomatic patients may refer to *C. trachomatis* infection.

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