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Quantitative Studies on the Role of *Ureaplasma urealyticum* in Non-Gonococcal Urethritis and Chronic Prostatitis

HELMUT BRUNNER M.D.,[a] WOLFGANG WEIDNER, M.D.,[b] AND HANS-GERD SCHIEFER, M.D.[c]

[a]*Professor of Medical Microbiology and Immunology, University of Düsseldorf, and Institute for Chemotherapy, Bayer AG, Wuppertal, Federal Republic of Germany;* [b]*Department of Urology, Justus Liebig University of Giessen, Federal Republic of Germany;* [c]*Professor of Medical Microbiology, Institute for Medical Microbiology, Justus Liebig University of Giessen, Federal Republic of Germany*

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Quantitative determinations of *U. urealyticum* and *M. hominis* have been performed in 164 men with non-gonococcal urethritis (NGU) and 597 patients with chronic prostatitis. Evidence is provided that *U. urealyticum* plays an etiologic role in 29.3 percent of patients with non-gonococcal urethritis. Mixed infections of *C. trachomatis* and *U. urealyticum,* in high numbers, do occur in 11 percent of NGU cases. A constellation suggesting ureaplasma-associated disease could be observed in 13.7 to 15.2 percent of 597 patients with chronic prostatitis. *M. hominis* does not appear to be a causative agent of NGU or chronic prostatitis.

INTRODUCTION

Non-gonococcal urethritis (NGU) appears to be the most frequent sexually transmitted disease in industrialized countries [1,2,3]. Approximately 50 percent of NGU cases are caused by *Chlamydia trachomatis* [3]. In addition, it has been well established that *Ureaplasma urealyticum* is another etiologic agent of NGU [3,4]. But, in an individual man with chlamydia-negative urethritis, it has been difficult to demonstrate that *U. urealyticum* is the causative pathogen, because up to 70 percent of healthy persons harbor the organisms in their urethra [3,4,5]. Titer rises of serum antibodies in NGU patients have not been demonstrated so far, either because of the insufficient sensitivity of the methods employed or because of the superficial nature of the infection, which does not induce high levels of systemic antibodies. In a different approach, we have therefore used quantitative procedures to establish the possibility of *U. urealyticum* as the etiologic agent in individual patients with NGU and thus determine the role of the organisms as a primary pathogen in this disease.

The etiology of chronic prostatitis is by far more obscure than that of NGU [6]. Common pathogens of the urogenital tract can only be found with low frequency (< 10 percent) in patients with chronic prostatitis. Because *U. urealyticum* can cause urethritis, the possibility arises that the organisms also induce ascending inflammatory reactions of the prostate. Since the technique of Meares and Stamey has suc-

Address reprint requests to: Prof. Dr. med. H. Brunner, Institute for Chemotherapy, P.O. Box 101709, Bayer AG 5600 Wuppertal-1, FRG

cessfully been used to localize infections of the male genital tract with common bacteria, we have employed this procedure, together with quantitative culture techniques, for common bacteria, candida species, *U. urealyticum,* and *Mycoplasma hominis* in order to unravel the role of the latter two species in chronic prostatitis [7]. This publication summarizes data on 164 patients with NGU and 597 men with chronic prostatitis [8,9].

STUDY POPULATION AND METHODS

Study Population

One hundred and sixty-four patients (mean age, 34 years), attending the outpatient clinic of the Department of Urology, University of Giessen, from January 1, 1979, until December 31, 1980, were studied. All men complained of spontaneous urethral discharge. The presence of *Neisseria gonorrhoeae* was excluded by gram stain and inoculation of discharge on Thayer-Martin agar.

In addition, 597 men with chronic prostatitis were examined during the period of 1975–1980. Patients with prostatitis typically complained of variable symptoms, like urinary frequency, urgency, nocturia, terminal dysuria, abdominal aching, backache, and testicular, penile, or perineal pain. In a few asymptomatic men prostatic tenderness or edema at rectal examination were the reasons for further diagnostic procedures. Thirty-two (5.4 percent) of these patients (mean age, 41 years) had chronic bacterial prostatitis with common urinary tract pathogens, predominantly *Escherichia coli.* Prostatodynia was the diagnosis in 187 men (31.3 percent) (mean age, 39 years). In 82 patients (mean age, 42 years) ureaplasma-associated prostatitis was suggested by quantitative culture criteria as detailed in results. In 296 men (49.6 percent) the etiology of the disease remained obscure. Men with upper urinary tract infection by known pathogens and patients who had taken antibiotics within four weeks before attendance were excluded from the study. Forty-eight men (mean age, 42.5 years) without inflammatory response of the urogenital tract were studied as controls. In addition, uroflowmetry was used for detection of obstructive disorders as a cause of recurrent infections.

Microbiological Procedures (Table 1)

In men with NGU, 0.01 ml of urethral discharge was taken with a bacteriological loop from the meatus urethrae and transferred to a transport medium [10]. In addition, fluor urethrae was inoculated into cycloheximide-treated McCoy cells for the cultivation of chlamydiae [11]. For isolation of ureaplasmas and mycoplasmas, 0.1 ml of the transport medium and 0.1 ml of first-voided urine were inoculated on A-6D agar, later A-7 agar, and into U-9 broth [12,13]. For cultivation of *M. hominis* a standard mycoplasma agar and broth were also inoculated. These two media were later omitted, when it was found that *M. hominis* could be isolated with the same efficiency on A-6-D, resp. A-7, agar and U-9 broth. After incubation of agar plates for five days under anaerobic conditions (Gas-Pak-System, Becton-Dickinson, Heidelberg, FRG) colonies were counted, using a dissecting microscope at a magnification of 25 ×. The U-9 broth was observed twice daily for a color change of the pH indicator. When the indicator started to change color, subcultivation was performed immediately on agar plates. If a color change was not seen after five days, the tubes were discarded. Ureaplasmas were identified by their characteristic colony morphology and by their resistance to lincomycin. *M. hominis* was identified by epi-immunofluorescence. Details of the procedures have been described previously [8–10].

TABLE 1
Diagnostic Schedules in Urethritis (164 men), Chronic Prostatitis (597 patients), and 48 Healthy Volunteers

Urethritis	Prostatitis and Controls
Urethral Discharge Quantitative culture for mycoplasmas Gram stain and culture for: *Neisseria gonorrhoeae* Culture for: *C. trachomatis*	VB_1: Quantitative culture for common bacteria, mycoplasmas, and fungi
VB_1: Quantitative culture for common bacteria, mycoplasmas, and fungi and microscopy + culture for *T. vaginalis* sediment: leucocytes	VB_2: as VB_1 and sediment: leucocytes
	EPS: as VB_1; Gram stain and culture for *N. gonorrhoeae,* culture for *C. trachomatis*
	VB_3: as VB_1; microscopy and culture for *T. vaginalis* sediment: leucocytes

In patients with prostatitis and in healthy controls, 10 ml of first-voided urine (VB_1), midstream urine (VB_2), expressed prostatic secretions (EPS), and 10 ml of first-voided urine after massage (VB_3) were obtained [7]. This procedure, proposed by Meares and Stamey, has proved to be very useful in localization of the infection. If midstream urine (VB_2) is sterile or contains very low numbers of bacteria and first-voided urine (VB_1) exhibits at least tenfold higher numbers of bacteria than EPS and VB_3, the infection is localized to the urethra (urethritis). In bacterial prostatitis, the number of CFU in EPS and usually also in VB_3 exceeds by at least one logarithm the number of CFU in VB_1. If VB_2 contains significant numbers of bacteria (10^5 CFU or more) the infection cannot be localized to the urethra or the prostate and is therefore considered as upper urinary tract infection.

Common urinary tract pathogens were quantitated, according to standard techniques. Quantitative culture of ureaplasmas and mycoplasmas in all four kinds of specimens was as described above. *C. trachomatis* was cultured in EPS. In addition, EPS was investigated by gram stain and culture on Thayer-Martin medium for the presence of *Neisseria gonorrhoeae.* Microscopy and cultivation for *Trichomonas vaginalis* was performed on VB_3 (Table 1). Patients with positive results for *N. gonorrhoeae, C. trachomatis,* or *T. vaginalis* have not been included in the study. Leucocyte counts could not be performed on EPS, because all available material was used for the microbiological procedures.

RESULTS

Non-Gonococcal Urethritis

As can be seen in Table 2, *U. urealyticum* could be isolated in numbers of more than 10^4 colony-forming units (CFU) from urethral discharge and more than 10^3 CFU per ml from VB_1 in 48 (29.3 percent) of the 164 NGU patients. In six of these 48 patients, mixed infections with *M. hominis,* also in high numbers, were observed. In 18 patients (11 percent) *U. urealyticum* in high numbers and *C. trachomatis* were isolated, whereas *C. trachomatis* alone was seen in 59 (36 percent) of men with NGU. Ten or more leucocytes per high-power field (400 ×) of the sediment from VB_1 were observed in 71.7 percent of chlamydia-positive, ureaplasma-negative men and 47.6 percent of the 42 patients with high ureaplasma numbers. Furthermore, *T.*

TABLE 2
Non-Gonococcal Urethritis
(n = 164)

Organisms Isolated	No. of Men	%
U. urealyticum[a]	42	25.6
U. urealyticum[a] + *M. hominis*[a]	6	3.7
U. urealyticum[a] + *C. trachomatis*	18	11
C. trachomatis	59	36
T. vaginalis	3	1.8

[a] $>10^4$ CFU/ml urethral discharge
$>10^3$ CFU/ml VB_1 (voided bladder 1)

vaginalis was detected in three (1.8 percent) of the 164 men with NGU. The patients, from whom *C. trachomatis* and high numbers of *U. urealyticum* had been isolated, were treated with 500 mg of tetracycline-HCl twice daily for 14 days. All men were cured two to three weeks after therapy.

Chronic Prostatitis

In 82 (13.7 percent) of the 597 patients with chronic prostatitis the typical prostatitis histogram, described by Meares and Stamey [7], was observed for *U. urealyticum* (Table 3). Eighty of these patients had high numbers of ureaplasmas in EPS and 51 also in VB_3 but low numbers or no ureaplasmas in VB_1 and VB_2. In the remaining two patients, who had high ureaplasma numbers in VB_1 and VB_2, CFU in

TABLE 3
Prostatitis Constellation for *U. urealyticum* (82 men) and *M. hominis* (10 men) of 597 Patients Studied with the Chronic Form of the Disease, in Accordance with the Proposal by Meares and Stamey for Localization of the Infection

Specimens from Which Indicated No. of Organisms Were Isolated	No. of Men	%
U. urealyticum		
VB_1: $>10^3$ CFU/ml	2	0.3
VB_2: $>10^3$ CFU/ml	1	0.2
EPS: $>10^3$ CFU/ml	82	13.7
VB_3: $>10^3$ CFU/ml	51	8.5
M. hominis		
VB_1: $>10^3$ CFU/ml	0	0
VB_2: $>10^3$ CFU/ml	0	0
EPS: $>10^3$ CFU/ml	10[a]	1.7
VB_3: $>10^3$ CFU/ml	3[a]	0.5

[a] Mixed infections with *U. urealyticum* (seven patients), *E. coli* (two patients) or *S. faecalis* (one man)

CFU, colony-forming units

VB_1, first-voided urine

VB_2, midstream urine

EPS, expressed prostatic secretions

VB_3, urine voided after prostatic massage

Prostatitis, high numbers of CFU in VB_3 and/or EPS, low numbers in VB_1 and VB_2

VB_1 and VB_2 were more than tenfold lower than in EPS, which was still in accordance with a prostatitis histogram. In an additional nine patients, high numbers of ureaplasmas were observed in VB_1, VB_2 (eight patients), and EPS (data not shown). In these men a significant difference in the number of organisms between VB_1 resp. VB_2 and EPS was not seen, indicating most probably prostato-urethritis. If these nine are added to the previous group of 82 men, a total of 91 (15.2 percent) of the patients may be considered to suffer from ureaplasma-associated chronic prostatitis.

The 82 men with ureaplasma-associated prostatitis were treated with 500 mg of tetracycline-HCl twice daily for 14 days. Seventy-one of these patients were cured 21 to 28 days after start of treatment, and ureaplasmas were no longer detected. Seven of the remaining eleven men had bladder neck obstruction. In four men no obvious explanation for the therapy failure could be found.

M. hominis was cultured from ten men in the typical prostatitis configuration, but always in mixed infections with high numbers of either *U. urealyticum* (seven men), *Escherichia coli* (two), or *Streptococcus faecalis* (one), who also showed a prostatitis pattern (Table 3). In an additional 12 patients, from whom *M. hominis* could be isolated in high numbers, a prostatitis histogram was not seen (data not shown). Eleven of these men had more than 10^3 CFU in VB_1, midstream-urine, and EPS.

U. urealyticum was isolated from VB_1 (eight men), VB_2 (none), EPS (three men), and VB_3 (three men) of the 48 healthy persons but always in numbers below 10^3 CFU per ml. *M. hominis* was cultured from VB_1 (six men), VB_2 (none), EPS (one man), and VB_3 (one man) of healthy volunteers. Although in one man more than 10^4 CFU of *M. hominis* per ml of EPS and $10^{3.7}$ CFU per ml of VB_3 were isolated, he did not exhibit any symptoms or signs of lower genital tract disease.

In 32 (5.4 percent) of the 597 men, common urinary tract pathogens caused the disease, whereas 187 (31.3 percent) patients suffered from prostatodynia. In the remaining patients variable results were obtained which did not permit an etiological diagnosis.

DISCUSSION

Our studies on non-gonococcal urethritis have confirmed previous findings, which indicated that approximately 45 to 50 percent of the NGU cases are caused by *C. trachomatis* [1,2,3]. In addition, evidence was provided that in approximately 30 percent of the NGU patients *U. urealyticum* was the etiologic agent. As expected, in 11 percent of patients a mixed infection of *C. trachomatis* and high numbers of *U. urealyticum* was observed. In these men the etiology remained obscure.

In previous studies on 312 patients with prostato-urethritis, it was demonstrated that disease symptoms in men, who had high numbers of *U. urealyticum* in EPS and VB_3, disappeared after tetracycline treatment, and the mycoplasmas could not be re-isolated, whereas in patients with high numbers of *M. hominis* disease symptoms persisted, although mycoplasmas were no longer detected after therapy [14]. The data indicated that *U. urealyticum,* but not *M. hominis,* was connected with disease. In further studies, tetracycline therapy was therefore considered to be justified only in patients with high numbers of *U. urealyticum.*

Ureaplasma-associated prostatitis was evident in 13.7 to 15.2 percent of men with chronic prostatitis. Therapy failure in 11 of these patients could be explained by bladder neck obstruction, reinfection via the sexual partner, or by appearance of tetracycline-resistant ureaplasmas [15]. In addition, infection with another tetracycline-sensitive microorganism should be considered [16]. The data do not ultimately prove the etiologic importance of *U. urealyticum* in these men, but isola-

tion and treatment results point in this direction. Further studies, using serum or local antibody determinations and/or serotyping of isolates could provide additional evidence for the causative role of the organisms in chronic prostatitis.

For the etiological diagnosis of NGU we regard it as mandatory to include culture procedures for *C. trachomatis* and quantitative (CFU) determinations of *U. urealyticum*. In chronic prostatitis, the localization technique of Meares and Stamey can now be recommended for the diagnosis of ureaplasma infection similar to its previous use for prostatitis caused by common bacteria.

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