Etiology of Nongonococcal Urethritis

EVIDENCE FOR *CHLAMYDIA TRACHOMATIS* AND *UREAPLASMA UREALYTICUM*

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A B S T R A C T *Chlamydia trachomatis, Ureaplasma urealyticum* (T-mycoplasma), and *Hemophilus vaginalis* have previously been considered possible etiological agents in nongonococcal urethritis (NGU). In this study, current *C. trachomatis* infection was confirmed by culture and (or) micro-immunofluorescence serology in 26 of 69 men experiencing a first episode of NGU, and 1 of 39 with no urethritis. Serum IgM immunofluorescent antibody to chlamydia was demonstrated in 16 of 20 men with chlamydia culture positive NGU, and 3 of 39 with chlamydia culture negative NGU, and none of 34 with no urethritis. 9 of 10 culture positive men with ≤10 days symptoms developed immunofluorescent antibody seroconversion in paired sera. *U. urealyticum* was isolated significantly more often and in significantly higher concentration from first voided urine from chlamydia-negative cases of NGU than from chlamydia-positive NGU. Ureaplasmacidal antibody titers increased fourfold in six men, four of whom had negative cultures for ureaplasma. *H. vaginalis* was isolated from 19 of 33 men with no urethritis and 2 of 69 with NGU. *C. trachomatis* is susceptible, and *U. urealyticum* is resistant to sulfonamides. A 10-day course of sulfisoxazole therapy produced improvement in 13 of 13 chlamydia-positive, ureaplasma-

negative, and only 14 of 29 chlamydia-negative, ureaplasma-positive NGU cases ($P < 0.002$). Thus, culture, serology, and response to therapy support the etiologic role of chlamydia in NGU. Quantitative culture and response to therapy suggest *U. urealyticum* may cause many cases of chlamydia-negative NGU.

INTRODUCTION

Chlamydia trachomatis has been recovered from the urethra from 30–50% of men with nongonococcal urethritis (NGU)[1] (1–4), and from a significantly lower proportion of controls without urethritis (1, 2), or with gonorrhea (1). Serologic evidence suggests chlamydia infection is recently acquired in many men with NGU (1, 5, 6). Chlamydia can be recovered from the endocervix of sex partners of most NGU patients with chlamydia infection, but seldom from sex partners of NGU patients without chlamydia infection (3). Persistence or recurrence of urethritis within 1–6 wk after completion of 7 days' therapy with tetracycline is significantly more common for chlamydia-negative NGU (47%) than for chlamydia-positive NGU (17%) (7).

The etiology of chlamydia-negative NGU has not been elucidated by previous qualitative analysis of the urethral flora of men with and without NGU (1), but *Ureaplasma urealyticum* (8) and *Hemophilus vaginalis* (9, 10) have been suspected to be possible causes of NGU. Previous studies indicate 22–90% of NGU cases respond to sulfonamide therapy (11–16).

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[1] *Abbreviations used in this paper*: CCU, color changing units; IFA, immunofluorescent antibody; NGU, nongonococcal urethritis.

Since *C. trachomatis* is sensitive to sulfonamide (17), while *U. urealyticum* (18) and *H. vaginalis* are resistant to sulfonamides (19), the present study was undertaken to compare the response of chlamydia-positive and chlamydia-negative NGU to sulfisoxazole. We reasoned that if chlamydia-positive NGU responded to sulfisoxazole while chlamydia-negative NGU did not, this would support the etiologic role of *C. trachomatis* in NGU and would suggest that the causative agent(s) of chlamydia-negative NGU was a sulfonamide-resistant organism. Quantitative cultures for *H. vaginalis* and *U. urealyticum* were performed in conjunction with the therapeutic trial, together with urethral cultures for *C. trachomatis*, and testing of paired sera for immunofluorescent antibody to *C. trachomatis* and complement-dependent ureaplasmacidal antibody to *U. urealyticum*. The prevalence of *H. vaginalis* and *U. urealyticum* was compared in men with chlamydia-positive NGU, chlamydia-negative NGU, and men with no urethritis. To minimize the occurrence of urethral colonization or serum antibody related to previous NGU, men who had previously had urethritis were excluded from this study.

METHODS

Study population. Caucasian males attending the Seattle King County Venereal Disease Clinic with complaints of discharge and (or) dysuria, and who had a demonstrable urethral exudate were accepted into this study if they were under 36 yr of age, had never had NGU or gonorrhea or symptoms suggestive of urethritis, had not taken antimicrobials in the preceding 3 mo, had three or less sex partners in the preceding 3 mo, had not had symptoms longer than 1 mo, were not allergic to sulfonamides, had negative Gram stain and culture of urethral exudate for *Neisseria gonorrhoeae*, and had pyuria demonstrated as described below. Men attending the same clinic who fulfilled the same criteria except that they had no symptoms and signs of urethritis or pyuria formed a "no urethritis" comparison group. Written consent was obtained from all men in the study.

Pretreatment evaluation. At the initial visit, men in both the NGU and the no urethritis groups underwent a standardized interview concerning demography, sexual history, venereal disease history, and present illness; examination of the genitalia, and inguinal lymph nodes; and careful inspection of the bulbar and palpebral conjunctiva.

Urethral exudate from NGU patients was examined by Gram stain and culture on Thayer-Martin medium to exclude gonorrhea. A calcium alginate urethrogenital swab was then inserted into the urethra 1–2 cm beyond the fossa navicularis and was placed into transport medium for isolation of *C. trachomatis*. Men with no urethritis underwent urethral culture and Gram stain to exclude gonococcal infection. All men were asked to return 1–3 days later, without having voided overnight.

At the second pretreatment visit, the men with no urethritis and the NGU patients all underwent the following studies. A urethral specimen was obtained for isolation of *C. trachomatis*, a second swab was inserted 1 cm further to obtain a urethral swab for Gram stain, and a third

swab was then introduced 1 cm further and was used to inoculate a chocolate agar plate for isolation of *N. gonorrhoeae* and *H. vaginalis*. The first 15 ml of overnight urine was then collected and the patient voided most of the remainder of the urine. A 0.01-ml portion of urine was directly inoculated onto a chocolate agar plate and a 3.0-ml portion was placed in a sterile tube, refrigerated for 2–3 h, then cultured qualitatively and quantitatively for *U. urealyticum*.

Approximately 7 ml of the urine was centrifuged at 500 g for 10 min. The supernate was discarded and the pellet resuspended in 0.5 ml of residual urine and examined by high dry microscopy (×400) for trichomonads and leukocytes. The presence of 20 or more polymorphonuclear leukocytes in at least two of five ×400 fields was considered to represent pyuria.

The prostate was then massaged until the patient felt fluid in the urethra. The next 15 ml of postprostatic massage urine was then collected. A 0.1-ml portion was placed in chlamydia transport media, a 0.01-ml portion was inoculated onto a chocolate agar plate, and the urine was examined for pyuria.

Therapy and follow-up examinations. NGU patients were given 500 mg of sulfisoxazole four times daily for 10 days, and were asked to return at 4, 10, 21, and 35 days after onset of therapy. At each follow-up visit, NGU patients were questioned about symptomatology and interim sexual activity, and were examined for urethral exudate. One urethral specimen was obtained for isolation of chlamydia, and a first-voided overnight urine was then obtained and examined for pyuria. If urethritis persisted, qualitative and quantitative cultures for *U. urealyticum* were repeated. Serum was collected at each visit.

Clinical response. Response to therapy at 7–12 days was classified as complete, partial, or no response, according to objective criteria. A complete response was total disappearance of discharge and pyuria. No response indicated no improvement in discharge and pyuria. Partial response included individuals whose discharge disappeared completely, but pyuria persisted; or whose discharge was decreasing and had become clear and nonpurulent, but had not completely disappeared. The assessment of discharge and pyuria was recorded by the clinician, who was unfamiliar with the results of the microbiological data. The subsequent grouping of patients into complete, partial, or no response categories on the basis of the clinician's recorded assessment was made by an individual who was not given the results of the microbiological data.

Bacteriologic methods. Transport medium for chlamydia isolation was frozen at −70°C until tested, usually within 2–3 wk. Chlamydia isolation was performed on iododeoxyuridine-treated McCoy cells as previously described (20). *U. urealyticum* isolation was performed on A-6 agar as described by Shepard and Howard (21), and in broth medium as previously described (1). Serial tenfold dilutions of the first voided urine were made in broth for quantitation of *U. urealyticum* in urine. The broth contained phenol red to detect increase in pH caused by the release of ammonia from urea, and the concentration of *U. urealyticum* was expressed as the number of color changing units, (CCU) per milliliter of urine.

The chocolate agar plates were immediately placed in a candle extinction jar and incubated at 37°C. Isolates identified as *H. vaginalis* were small gram negative bacilli showing "diphtheroid" arrangements on Gram stain and producing small, entire, raised, greyish colonies on chocolate agar after 48 h incubation (22). All isolates grew poorly or not at all on sheep's blood agar, and were catalase and oxidase negative. In peptone-water base with 10% added fetal calf serum, all

isolates fermented glucose and maltose, none fermented lactose, and 25% fermented sucrose. Glucose fermentation products were determined by gas chromatographic analyses of 48-h cultures in peptone-yeast-glucose broth containing 10% fetal calf serum (23). All isolates produced large amounts of acetic acid as their only major fermentation product. 40% also produced a small amount of lactic acid.

Description of serologic methods. Sera were stored at −20°C until tested. Tests were carried out without previous knowledge of the results of other studies for these patients. Three fourfold dilutions of serum (1:8, 1:32, and 1:128) were tested in the simplified microimmunofluorescence test (24) against a set of *C. trachomatis* antigens including immunotypes A, CJ, B, DE, FG, K, H, and I. All paired sera were first screened for antibody with commercial fluorescein conjugates of antihuman immunoglobulins and anti-IgM (μm-chain specific). Selected early sera with positive antibody were further tested concurrently for both IgM and IgG antibodies with commercial fluorescein conjugates of antihuman IgM (μm-chain specific) and of antihuman IgG (γ-chain specific). Antibody titer was expressed by the reciprocal of the highest serum dilution which resulted in definite immunofluorescence, a titer of <8 was considered a negative reaction. Seroconversion or a titer rise of fourfold or greater in paired sera was considered significant.

Complement-dependent mycoplasmacidal antibody to 11 strains of *U. urealyticum* was measured as previously described (25), except that the serum was not dialyzed before use.

Quantitative determination of antibody to gonococcal pili was performed as previously described (26), except that protein A containing staphylococcus was used in the place of the second antibody to human IgG. With this modification, we have found serum antibody concentrations ≥ 1 $\mu g/ml$ to be correlated with the presence of gonococcal infection.

Statistical analysis. The proportions of groups affected by selected variables were compared with Fisher's exact test (27) with $n \leq 60$ and Chi square analysis with Yates correction (28) with $n > 60$. Mean and standard deviation of series of data were compared by Student's t test (29).

RESULTS

69 men with NGU and 39 men with no urethritis were studied. 13 of those with no urethritis had other sexually transmitted genital diseases, while 26 attended the clinic for routine examination. Men with NGU were similar to those with no urethritis with respect to mean years of age±1 SD (23.0±4.1 vs. 23.2±3.6), mean years of education (14.0±1.7 vs. 14.4±2.0), and mean age of first intercourse (17.2 ±2.3 vs. 17.8±2.6, $P > 0.20$). Those with NGU had a lower median number of total sex partners than those with no urethritis (4.8 vs. 8.0). 41 of 67 NGU patients and 11 of 35 men with no urethritis had ≤ 5 total sex partners ($X^2 = 5.87$, $P < 0.025$).

C. trachomatis isolation and serology. *C. trachomatis* was recovered from the urethra of 23 (33%) of 69 NGU patients and 1 of the 39 with no urethritis ($P < 0.001$). *C. trachomatis* was recovered from 4 (6%) of 69 postprostatic massage urine specimens from NGU patients, and all 4 had concomitantly positive urethral cultures. Postprostatic massage urine cultures from 32
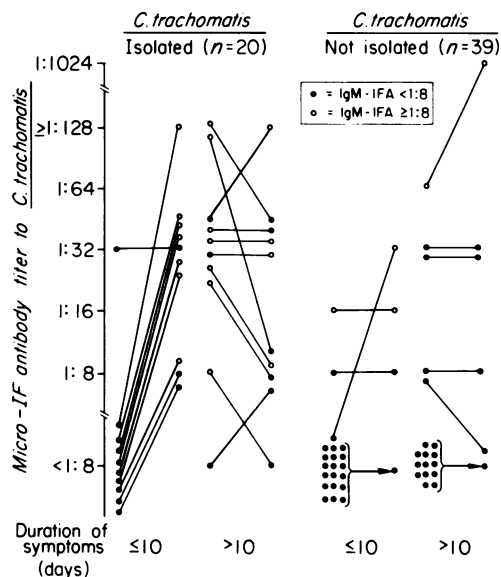

FIGURE 1   Micro-IFA titers to *C. trachomatis* in men with *C. trachomatis* culture-positive and *C. trachomatis* culture-negative NGU. Each group is subdivided according to whether symptoms were present ≤ 10 days, or >10 days. Acute and convalescent sera for each patient are connected by a solid line.

men with no urethritis were all negative for *C. trachomatis*. NGU patients with and without chlamydia isolation were similar with respect to mean age, mean years of education, mean age of first intercourse, and median number of lifetime sex contacts.

Paired sera were collected from 20 chlamydia-culture-positive patients and 39 chlamydia-culture-negative patients. The results of the micro-immuno-fluorescent antibody (IFA) test with *C. trachomatis* in the two sera are shown in Fig. 1 according to presence or absence of chlamydia by culture and the duration of symptoms. All 20 chlamydia-culture-positive men had IFA titers $\geq 1:8$. Of those with symptoms of ≤ 10 days duration, 9 of 10 seroconverted from no detectable antibody in the initial serum to titer $\geq 1:8$ in the convalescent serum and 1 showed a stable titer. Of the 10 with symptoms > 10 days, 1 seroconverted, 1 had a fourfold rise, 3 had stable titers, and 5 had falling titers. Of the 39 men from whom chlamydia were not isolated from the urethra, 1 seroconverted, 1 had a fourfold rise in titer, 5 had stable titers, 1 had a falling titer, and 31 did not develop detectable IFA.

The remaining 10 patients with NGU not shown in Fig. 1 had only a single serum tested. None of three from whom chlamydia were isolated had detectable IFA, and one of seven chlamydia-culture-negative patients had detectable IFA (titer 1:8). 5 of 34 men with no urethritis tested had micro-IFA on the single sample tested.

	NGU		No urethritis	
	Chlamydia positive	Chlamydia negative	Chlamydia positive	Chlamydia negative
H. vaginalis	2/26	1/43	0/0	19/33
U. urealyticum	11/26	35/43	1/1	22/38

Fig. 1 also depicts sera with IgM-IFA to *C. trachomatis* detected at ≥1:8 as open circles, and sera without detectable IgM-IFA as solid circles. IgM-IFA was detected in 16 of the 20 seropositive, culture-positive patients, including 7 of the 10 seroconverters, the 1 with a fourfold rise, and 8 of the 9 with stable or falling titers. Of the nine seropositive chlamydia-culture-negative men, it was detected in three. These were the one seroconverter, the one with a fourfold increase in the micro-IFA, and one of five with a stable micro-IFA titer.

22 of the 23 isolates were typed. There were 16 DE, 4 FG, 1 CJ, and 1 I immunotypes. In 17 of the 19 instances in which the isolate was typed and serum IFA was detected, the immunotype pattern of serum IgM-IFA corresponded to the immunotype isolated. In one case, the IgM-IFA was to immunotype FG, whereas the immunotype isolated was DE. In another, the highest IgM-IFA titer was to K, whereas the isolate was DE.

For purposes of this paper, men from whom chlamydia were cultured or who had a fourfold or greater rise or seroconversion of IFA, or had IgM-IFA demonstrated, will be called chlamydia-positive. The remaining patients will be called chlamydia-negative. Thus, among 23 chlamydia positive patients from whom paired sera and culture were obtained, *C. trachomatis* infection was confirmed in 19 by both culture and serology, in 1 by culture alone, and in 3 by serology alone. Three additional patients from whom no convalescent serum was obtained were positive by culture alone.

H. vaginalis isolation. As shown in Table I, *H. vaginalis* was isolated significantly more often from men with no urethritis than from men with chlamydia-positive NGU (*P* < 0.001), or chlamydia-negative NGU (*P* < 0.001). Among 22 men from whom *H. vaginalis* was isolated, this organism was recovered from the urethral swab from 95% of patients, from first voided urine from 86%, and from the postprostatic massage urine from 59%.

U. urealyticum isolation and serology. *U. urealyticum* was the only agent found to be significantly associated with chlamydia-negative NGU. *U. urealyt-*

icum was recovered from the first voided urine from a higher proportion of chlamydia-negative NGU patients (35 [81%] of 43), than from either chlamydia-positive NGU patients (11 [42%] of 26), (*P* < 0.005), or from men with no urethritis (23 [59%] of 39), (*P* < 0.05).

The results of the quantitative cultures for *U. urealyticum* in the first voided urine are shown in Table II. *U. urealyticum* was isolated in a concentration of ≥ 10³ CCU/ml first voided urine from 22 (52%) of 42 men with *C. trachomatis*-negative NGU, three (12%) of 26 with *C. trachomatis*-positive NGU (*P* < 0.005), and 8 (21%) of 39 with no urethritis (*P* < 0.025).

The difference between chlamydia-negative NGU patients and men with no urethritis, in terms of qualitative isolations of *U. urealyticum*, is greater if patients are grouped or matched according to their total number of sex partners. Among men who had had intercourse with 5 women or less during their lifetime, *U. urealyticum* was recovered from 22 (92%) of 24 with chlamydia-negative NGU and 3 (23%) of 13 with no urethritis (*P* < 0.002) and 8 (50%) of 16 with chlamydia-positive NGU (*P* < 0.01). It was also possible to consecutively match, in the order that they were admitted to the study, 20 chlamydia-negative NGU patients with 20 patients with no urethritis exactly for total number of lifetime sex contacts and for age ±2 yr. *U. urealyticum* was recovered from 17 of 20 chlamydia-negative NGU patients and from 9 of 20 matched controls with no urethritis (*P* < 0.02).

Sera obtained from men receiving antimicrobials active against ureaplasma (e.g. tetracycline or spectinomycin) were excluded from the studies of ureaplasmacidal antibody. A fourfold rise or fall in titer of complement-dependent ureaplasmacidal antibody was de-

	NGU		Control
U. urealyticum CCU/ml*	Chlamydia positive	Chlamydia negative	No urethritis
0	15	8	16
10^1	5	4	6
10^2	3	8	7
10^3	1	4	5
10^4	—	11	3
10^5	—	6	—
≥10^6	2	1	—

* CCU/ml, color changing units per milliliter first voided urine.

TABLE III
Ureaplasmacidal Antibody in Paired Sera in Relation to C. Trachomatis (C) and U. Urealyticum (U)

	C+U+	C+U−	C−U+	C−U−
Fourfold change				
with complement	1	2	1	2
without complement	—	—	5	—
Stable titer				
≥1:4	2	—	1	2
<1:4	8	7	21	4

tected in 6 of 59 men (Table III.) Three were chlamydia-positive, and three chlamydia-negative. Four were ureaplasma-negative and two ureaplasma-positive. Another five, all chlamydia-negative, ureaplasma-positive, had fourfold changes in titers in the absence of complement. Five additional men, two of whom were ureaplasma-negative, had stable titers ≥1:4.

N. gonorrhoeae isolation and serology. Gram negative intracellular diplococci were not seen in smears of any urethral exudate, which was examined at both the first and second pretreatment visits. Cultures of urethral exudate on both Thayer-Martin and chocolate agar media were all negative for *N. gonorrhoeae.* Paired sera from 55 men were tested for antipili antibody. Antipili antibody was unmeasurable in either serum from 27 of 69 men including 7 of 26 chlamydia positive and 20 of 43 chlamydia negative (P = NS). In the remaining 42 men, with measurable antibody, none had ≥1.0 μg antibody/1 ml of serum in either specimen. The antipili antibody concentration ±1 SD was 0.36 ± 0.14 μg/ml for 19 chlamydia positive men and 0.33 ± 0.11 μg/ml for 24 chlamydia negative men (P < NS). None of the paired sera demonstrated a twofold rise in antibody concentration.

Clinical findings in relation to chlamydia and ureaplasma infection. Urethral discharge was scored on the basis of amount (spontaneous 3, easily expressible 2, expressed with difficulty 1) and purulence (yellow 3, white 2, clear 1). A total score of ≥4 was recorded before treatment for 8 of 15 patients who were chlamydia-positive and ureaplasma-negative, and for 30 of 34 who were chlamydia-negative, ureaplasma-positive (P < 0.05).

Pyuria was recorded as number of ×400 fields with ≥20 leukocytes/field. 6 of 15 chlamydia positive, ureaplasma negative men, and 21 of 35 chlamydia negative, ureaplasma positive men had ≥20 leukocytes in all five fields examined (P = NS).

Results of sulfisoxazole therapy. Sulfisoxazole was effective in eradicating chlamydia. Urethral cultures obtained 3–8 days after starting therapy were negative for chlamydia in all 22 intitially chlamydia-cul-

ture-positive men followed. In contrast, *U. urealyticum* was recovered at 7–12 days from 22 (92%) of 24 patients who were initially ureaplasma-positive, and from 2 (14%) of 14 who were initially ureaplasma-negative before treatment.

The results of sulfisoxazole therapy were correlated with the chlamydia status by culture and serology and with the pretreatment cultures for ureaplasma. As shown in Table IV, a complete or partial resolution of objective signs of urethritis was seen at 7–12 days in 21 (88%) of 24 chlamydia-positive and 18 (49%) of 37 chlamydia-negative patients (P < 0.01). The three chlamydia-positive patients who showed no response included both of the patients who had ≥10^6 CCU/ml of *U. urealyticum* and one with 10^1 CCU/ml. A complete or partial response was seen in 22 (54%) of 41 ureaplasma-positive and 17 (85%) of 20 ureaplasma-negative patients (P < 0.05).

A complete or partial response was observed in 13 (100%) of 13 chlamydia-positive, ureaplasma-negative cases and 14 (47%) of 30 chlamydia-negative, ureaplasma-positive cases (P < 0.002).

A high rate of recurrence of chlamydia-negative sulfonamide non-responsive NGU was noted after subsequent tetracycline therapy. 20 patients who showed no objective response, and 10 who showed a partial response after 10 days therapy with sulfisoxazole were treated with tetracycline 500 mg four times daily for 7 days. 29 returned for followup at the end of 1 wk of tetracycline therapy and all were significantly improved. Within the next 4 wk, 12 of 17 (71%) sulfonamide failures, and 4 of 9 who had responded partially to sulfonamide required further retreatment after the course of tetracycline because of recurrence of urethral discharge and (or) pyuria. At the time of initiation of the second retreatment, seven who had originally been ureaplasma positive were again recultured and ureaplasma was again recovered from five of the seven.

DISCUSSION

This study strongly supports the etiologic significance of *C. trachomatis* in NGU. Richmond et al. have postulated that some other agent may initiate NGU and

TABLE IV
Response of NGU 7–12 Days after Onset of Sulfisoxazole Therapy in Relation to C. Trachomatis (C) and U Urealyticum (U)

Objective response	C+U+	C+U−	C−U+	C−U−
Complete	4	4	3	1
Partial	4	9	11	3
None	3	0	16	3
	11	13	30	7

reactivate a latent chlamydial infection (2). If this were true, then *C. trachomatis* would be present coincidentally or as a secondary pathogen. The results of the micro-IFA testing, and of sulfonamide therapy in this present study support a primary etiologic role for *C. trachomatis*, at least in men with first episode NGU. Previous studies by Wang and Grayston with experimental ocular infection in Taiwan monkeys (*Macaca cyclopis*) showed that IgM-IFA was detectable only after primary infection, and disappeared within 5 wk after onset of infection in four of five monkeys. IgM-IFA did not reappear after rechallenge with the same immunotype (30). Therefore, the demonstration of seroconversion in 90% of culture-positive men examined within 10 days of onset of symptoms, and of IgM-IFA in 16 of 20 culture-positive men with first episode NGU suggests these men have had recent onset of chlamydial infection.

This study was particularly designed not only to test the importance of chlamydia in NGU, but also to elucidate a cause for cases of NGU in which chlamydia are not isolated. The serologic testing showed that 3 of 39 (8%) of the culture-negative men with paired sera probably had *C. trachomatis* as the cause of the NGU. Another 5 of the 39 chlamydia-culture-negative men with paired sera, and 1 more with a single serum had IFA detected without IgM-IFA. Five of the latter six men were followed after sulfonamide therapy and behaved like chlamydia-negative patients in that three showed no response and two showed a partial response to therapy. Despite careful questioning to exclude men with any history suggestive of previous urethritis, IFA to *C. trachomatis* was also detected in 5 of the 35 men in the comparison group. These seropositive men may have had an asymptomatic episode of chlamydial urethral infection or ocular chlamydia infection in the past. The ratio of nonsymptomatic/symptomatic urethral chlamydial infections is not known, but these data suggest that asymptomatic infections do occur.

The results of sulfisoxazole therapy suggest that most cases of chlamydia-negative NGU were caused by a sulfonamide resistant organism. Handsfield et al. previously showed that 4 (31%) of 13 men with chlamydia-culture-negative NGU had resolution or improvement in urethral discharge and pyuria within 0–3 days after completion of 1 wk therapy with placebo (7). Thus, sulfonamide was not much more effective than placebo for chlamydia-negative NGU.

No evidence for urethral gonococcal infection was provided by duplicate Gram-stained smears, cultures on two media, or by serologic testing for antipili antibody.

Urethral infection with *U. urealyticum*, but not with *H. vaginalis*, was associated with chlamydia-negative NGU. Since the initial discovery of *U. urealyticum*

by Shepard in 1954 (31), the role of this organism in NGU has been debated, as summarized by McCormack et al. (8). In the present study, the demonstration of an association between urethral *U. urealyticum* infection and NGU may be attributable to, (1) selection of patients with no past history of urethritis, (2) separation of cases of NGU into chlamydia-positive and chlamydia-negative groups, (3) quantitative cultures for *U. urealyticum*, (4) matching of patients with chlamydia-negative NGU with patients having no urethritis with respect to age, race and total number of sex partners, and (5) separate analysis of patients who had had ≤5 sex partners.

It should be noted that the differences in ureaplasma isolation between men with chlamydia-negative NGU and men with no urethritis could be explained if first voided urine specimens are less sensitive in detecting urethral ureaplasma infection in men with no urethritis than in men with urethritis, as has been suggested by Tarr et al. (32). However, the potential objection should not account for the difference observed between men with urethritis, in this case the chlamydia positive and chlamydia negative NGU groups. We have not found a difference between isolation techniques in other studies on 81 men with urethritis who had both an endourethral swab and a first voided urine obtained for ureaplasma culture; we found ureaplasma in both cultures in 52, in the swab alone in 2, and the urine alone in 3.

If there is an increase in isolation of ureaplasma with an increase in pyuria, and if chlamydia negative men have more pyuria, this could possibly account for some portion of the increased isolatism rate of ureaplasma in chlamydia-negative men. Chlamydia-negative men did not have significantly more pyuria than chlamydia-positive men in this study, but Holmes et al. (1) have previously demonstrated the association when a larger group of men was studied.

In a previous study in Seattle, *U. urealyticum* was not recovered more often from men with chlamydia-negative NGU than from those with chlamydia-positive NGU or with no urethritis. In that study, however, patients with prior urethritis or many sex partners were not excluded, and quantitative mycoplasma cultures were not employed (1). If *U. urealyticum* causes NGU, it is necessary to explain why these organisms are so often recovered from sexually active men with no urethritis. Perhaps *U. urealyticum* produces urethritis only in certain individuals, but in most is carried chronically without causing inflammation. Multiple serotypes of *U. urealyticum* have been demonstrated by metabolic-inhibition, mycoplasmacidal, and other serological tests (18). It is possible that only certain pathogenic strains are associated wth NGU, or that only the initial infection with any new strain could produce urethritis in a susceptible individual.

Any of the above possibilities could explain why an association of *U. urealyticum* with chlamydia-negative NGU was best demonstrated for individuals with fewest total number of sex partners. Such individuals would be most likely to be encountering *U. urealyticum* for the first time, since the prevalence of urethral *U. urealyticum* infection in men is correlated with total number of previous sex partners (33).

Previous therapeutic trials have shown that the tetracyclines and erythromycin are effective in the treatment of NGU (34, 35), while lincomycin (36) and the penicillins (11, 37, 38) are not. These results are consistent with either chlamydia or ureaplasma etiology, since both *C. trachomatis* and *U. urealyticum* are high susceptible to tetracycline and erythromycin but are relatively resistant to the penicillins and lincomycin (17, 18, 39, 40) in vitro. The therapeutic response to sulfisoxazole in the present study is consistent with separate chlamydia and ureaplasma etiologies. Ureaplasma is susceptible in vitro to spectinomycin (41) which has only slight activity against *C. trachomatis*. In another study (42), we have found that spectinomycin is significantly more effective for treatment of ureaplasma-positive, chlamydia-negative NGU than for ureaplasma-negative, chlamydia-positive NGU. In that study only 1 of 14 men given spectinomycin for chlamydia-positive NGU was improved 7–14 days after starting therapy. Furthermore, Handsfield et al. (7) found that only 1 of 10 men with chlamydia isolation positive NGU improved within 7–10 days during placebo therapy. Thus, the improvement of chlamydia positive NGU in 21 of 24 men given sulfisoxazole in the present study cannot be attributed to the natural course of the disease.

The present study establishes an association of *U. urealyticum* only with initial episodes of NGU, and does not prove the association is causal. Previous serologic studies have generally failed to show rises in serum antibody titers to ureaplasma in men with NGU (43–45). In the present study, even among men with high titers of ureaplasma in first voided urine, a ureaplasmacidal antibody response was uncommon. Further support for ureplasma etiology could be sought by cohort study, experimental inoculation, and perhaps by new serologic techniques.

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