

Role of *Chlamydia trachomatis* in non-acute prostatitis

P.-A. MÅRDH,* K. T. RIPA,* S. COLLEEN,† J. D. TREHARNE,‡
AND S. DAROUGAR‡

From the *Institute of Medical Microbiology, University of Lund; †Department of Urology, University Hospital, Lund; and ‡Virus Laboratory and WHO Collaborating Centre for Reference and Research on Trachoma and other Chlamydial Infections, Department of Clinical Ophthalmology, Institute of Ophthalmology, University of London

SUMMARY The possible role of *Chlamydia trachomatis* in non-acute prostatitis was investigated by cultural and serological techniques in a study of 53 adult males. *C. trachomatis* was isolated from the urethra of only one of the 53 patients and from none of the 28 specimens of prostatic fluid from the same patients. By means of a modified microimmunofluorescent test, serum chlamydial IgG antibodies at a titre of 1/64 or greater, or IgM antibodies at a titre of 1/8 or greater, or both were detected in six of the patients, suggesting a recent or current chlamydial infection, while IgG or IgA antibodies at a titre of 1/8 or greater were detected in the specimens of prostatic fluid from two of the 28 men studied. In the seven patients with evidence of chlamydial infection, as well as in a further 13 of the 53 patients studied, the presenting symptoms suggested non-gonococcal urethritis (NGU) rather than prostatitis. Thus in this study *C. trachomatis* would appear to play a minor aetiological role, if any, in non-acute prostatitis.

Introduction

In contrast to the rare condition of 'acute bacterial prostatitis', which is associated with recurrent urinary tract infections and is generally caused by bacteria belonging to the family *Enterobacteriaceae* (Meares and Stamey, 1968), the aetiology of the comparatively common condition of non-acute prostatitis is, except in a few cases, still not known (Colleen and Mårdh, 1975a).

Chlamydia trachomatis has recently been established as a frequent cause of sexually transmitted diseases (Oriel *et al.*, 1976; Alani *et al.*, 1977; Bowie *et al.*, 1977; Mårdh *et al.*, 1977; Rees *et al.*, 1977; Ridgway and Oriel, 1977; Woolfitt and Watt, 1977; Ripa *et al.*, 1978a, 1978b). The organism has been found in 30-70% of all cases of non-gonococcal urethritis (NGU), a condition which is probably more common than gonococcal urethritis (Wiesner, 1977). *C. trachomatis* has also been associated with acute epididymitis (Harnisch *et al.*, 1977; Berger *et al.*, 1978).

The present study was undertaken to investigate the presence of genital chlamydial infection in patients with symptoms of non-acute prostatitis using cultural and serological methods.

Material and methods

STUDY POPULATION

The study comprised 53 men, with a mean age of 32 years (standard deviation 8.7), and a range of 22-58 years. Of these patients, 50 had presented because of symptoms of non-acute prostatitis, that is, dull pain over the bladder which often spread down to the groins, to the testicles, the lower back, the perineum, and along the inner aspects of the thighs. In addition, symptoms of slight urethral obstruction and discharge were commonly found. Generally these patients had had symptoms intermittently for many years (mean 3.5 years; standard deviation 3.6; range 0.5-18 years). Three of the 53 patients were diagnosed as having non-acute prostatitis when investigated for infertility. Of these three, none had had symptoms of urogenital tract infection. Twenty of the patients had first presented with episodes of urethritis; in eight of these, gonococci were isolated at that time. In a further patient

Address for reprints: Dr P.-A. Mårdh, Institute of Medical Microbiology, Sölvegatan 23, S-223 62 Lund, Sweden

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the symptoms of prostatitis had started following an episode of epididymitis; neither gonococci nor established urinary tract pathogens were isolated at that time. Furthermore, one patient had had a slight urethral discharge and polyarthrititis which had persisted for two years. None of the 53 patients studied showed signs of urinary tract infection, that is, bacteriuria or pyuria or both.

In addition to the history and clinical findings the diagnosis of non-acute prostatitis was based on the presence of more than 15 leucocytes per $\times 45$ microscopic field in expressed prostatic fluid (Colleen and Mårdh, 1975a).

Of the 53 patients, 28 had completed their last course of antibiotic treatment (tetracyclines were generally used) more than six months before taking part in the study, and 20 between one and six months. Of the remaining five patients, four were taking tetracyclines when studied and one had finished treatment one week before examination.

SAMPLING AND ISOLATION TECHNIQUES

Urethral specimens for the culture of *C. trachomatis* and *Neisseria gonorrhoeae* were obtained from all 53 patients. The samples were collected and transported as previously described (Ripa *et al.*, 1978a). Sufficient expressed prostatic fluid was obtained to carry out cultures for *C. trachomatis* and *N. gonorrhoeae* in 28 patients. The isolation methods used for *N. gonorrhoeae* (Mårdh *et al.*, 1978) and *C. trachomatis* (Ripa and Mårdh, 1977) were those reported elsewhere.

Serum specimens were collected from 50 of the patients, which included 25 of the 28 patients from whom prostatic fluid had also been obtained. Both serum and prostatic fluid specimens were used for the serological tests.

SEROLOGICAL TESTS

The presence of antibodies to *C. trachomatis* in sera and prostatic fluid was determined by a modified microimmunofluorescence (micro-IF) test (Treharne *et al.*, 1977a). Four pools of chlamydial antigens were used: pool 1 contained TRIC agent, serotypes A, B, and C; pool 2 TRIC agent, serotypes D, E, F, G, H, I, and K; pool 3 LGV serotypes 1, 2, and 3; while pool 4 contained antigens prepared from selected strains of *Chlamydia psittaci* (Treharne *et al.*, 1977b).

The titres of IgG and IgM antibodies to these pools of antigens were determined in the serum specimens, and those of IgG and IgA antibodies to the same antigens in the specimens of prostatic fluid. The lowest serum dilution used for testing IgG antibodies in serum was 1/16, while that for IgM in serum and IgG and IgA in prostatic fluid

was 1/8. The presence of IgM antibodies at a titre of 1/8 or greater or IgG antibodies at a titre of 1/64 or greater, or both, in serum specimens was considered as evidence of active chlamydial infection.

Results

ISOLATION STUDIES

C. trachomatis was isolated from the urethra of one of the 53 patients. In three of the patients the cell cultures were destroyed by a nonspecific cytopathogenic effect, and the presence of chlamydiae could therefore not be established. The chlamydial cultures of the 28 prostatic fluid specimens were spoiled in one instance, and *C. trachomatis* could not be isolated in any of the remaining 27 specimens. *N. gonorrhoeae* was not isolated from any of the 53 patients.

SEROLOGICAL TESTS

The results of the micro-IF tests for IgG antibodies to *C. trachomatis* in the 50 serum specimens are shown in the Table. Four of the specimens contained these antibodies at a titre of 1/64 or greater, three of them reacting with the pool 2 antigens and the fourth with the antigens of both pool 2 and 3. IgM antibodies to *C. trachomatis* were found in four serum specimens, the titres being 1/8 in two specimens and 1/16 and 1/32 in each of the other two. Two of the specimens with chlamydial antibodies reacted with the antigens of both pool 2 and 3, while the other two reacted only with the pool 2 antigens. None of the 50 specimens contained antibodies to the *C. psittaci* antigens included in the test.

Of the 28 prostatic fluid specimens one gave an IgG antibody titre of 1/32 and one an IgG antibody titre of 1/16 and an IgA antibody titre of 1/8. The IgG antibodies in the two specimens reacted with the pool 2 and 3 antigens.

The patient with IgA antibodies to *C. trachomatis* in prostatic fluid at a titre of 1/32 had a serum IgG titre of 1/32 and an IgM titre of 1/8. A specimen of

Table Numbers of patients with non-acute prostatitis with reciprocal titres of type-specific chlamydial antibodies detected by an immunofluorescence test

Serum antibodies	Antibody titres							Total no. of patients
	<8	8	16	32	64	128	256	
IgG	43	ND	0	3*	1†	1‡	2§	50
IgM	46	2	1	1	0	0	0	50

ND Not done

* IgM antibody titre 1/8 in two patients

† Only patient culture-positive for *C. trachomatis*

‡ IgM antibody titre 1/16

§ IgM antibody titre 1/32 in one patient

serum was not obtained from the other patient in whom antibodies to *C. trachomatis* were found in the prostatic secretion. Prostatic fluid from another patient with serum IgG antibodies at a titre of 1/128 and IgM antibodies at a titre of 1/16 did not contain any detectable chlamydial antibodies.

CORRELATION OF CLINICAL FINDINGS WITH CULTURAL AND SEROLOGICAL TESTS

The clinical signs and symptoms in six of the seven patients with antibodies in serum or prostatic fluid or both, indicating active chlamydial infection, did not differ from those in the other 46 patients. The remaining seropositive patient had polyarthritides resembling Reiter's syndrome. All seven patients with antibodies first presented with NGU, as did a further 13 of the 53 patients.

The patient who had previously had an acute episode of epididymitis had chlamydial antibodies in both serum and prostatic fluid specimens. In the entire series of patients, eight of whom had previously had gonorrhoea, four had chlamydial antibodies.

The only patient with positive results to cultural and serological tests had finished three-weeks' doxycycline treatment one week before sampling. Of the remaining six patients with chlamydial antibodies three had been given tetracycline one to four months previously, and another two patients had had tetracycline two years previously. The patient with polyarthritides had never received antibiotic treatment.

Three of the seven patients with serological evidence of chlamydial infection had, as discovered retrospectively, been given doxycycline after sampling (100 mg daily for three weeks) and had become symptomless for some months. However, recurrent episodes or reinfections occurred later. This contrasted with the remaining three seropositive patients who were not given antibiotics and in whom the symptoms persisted.

Discussion

C. trachomatis is an established cause of acute urethritis in men. The organism may invade the accessory genital glands as demonstrated in patients with acute epididymitis in whom specimens collected from the epididymis by aspiration were culture-positive for *C. trachomatis* (Berger *et al.*, 1978). This might also suggest an aetiological role of *C. trachomatis* in prostatitis.

The frequency of isolation of *C. trachomatis* in the present series of patients with non-acute prostatitis (one out of 53 patients) is very low compared with that in a series of consecutive male patients (24.6% or 222 out of 902) from the same hospital

catchment region attending a venereal diseases clinic because of symptoms of acute urethritis (Mårdh *et al.*, unpublished data).

The patients with non-acute prostatitis had generally had their disease for several years. These patients may have harboured *Chlamydia* which was possible to detect by cultural methods at an early stage of the disease. The occurrence of chlamydial antibodies in the urethral secretion and expressed prostatic fluid, or a cellular immune response, or both might have interfered with the isolation of *Chlamydia* in the non-acute condition studied. Thus there may be an analogous situation with that of ocular hyperendemic trachoma, where it is possible to isolate *C. trachomatis* from 70% of acute cases but from only 5% of chronic (stage III-IV), complicated trachoma cases (Darougar *et al.*, 1977).

Contamination of the prostatic fluid with the contents of the urethra is a problem in aetiological studies of inflammatory conditions of the prostate, such as non-acute prostatitis. The use of prostatic biopsy specimens for culture to isolate an infectious agent in non-acute prostatitis is hampered by the risks involved in taking such specimens (Esposti *et al.*, 1975). Thus, serodiagnostic methods may prove more valuable in aetiological studies of non-acute prostatitis.

In an earlier study (Mårdh *et al.*, 1972) we used a complement fixation test (CF) to detect antibodies to *Chlamydia* in patients with non-acute prostatitis. Of the 79 patients studied 33% had CF antibodies at a titre of 1/5 or greater compared with 3% of 72 blood donors matched for age. We thought it worthwhile to investigate again patients with non-acute prostatitis for chlamydial antibodies, this time using the more recently developed, highly specific micro-IF technique. With this serodiagnostic technique we found that 12% of the 50 patients in the present series had evidence of a recent or current infection with *C. trachomatis* (IgM antibodies at a titre of 1/8 or greater, or IgG antibodies at a titre of 1/64 or greater, or both). However, if a titre of 1/16 for IgG chlamydial antibodies is included one further patient of the 53 studied would be considered seropositive. Chlamydial antibodies were found in the prostatic secretions in only two of the 28 patients.

By means of the micro-IF technique, 51% of men with NGU and positive cultures for *C. trachomatis* had IgG antibodies at a titre of 1/8 or greater. In men with NGU but negative cultures for *Chlamydia* the corresponding figure was 10% (Treharne *et al.*, 1977b).

In another study, we found no evidence of bacteria as the cause of non-acute prostatitis

except in a few cases. Using an immunofluorescent technique we found gonococci in prostatic fluid specimens in about 10% of the 79 patients studied, but it was not possible to isolate the bacteria from these patients. *Mycoplasma hominis* was cultured from 10% of the patients but from none of the 20 controls studied, while *Ureaplasma urealyticum* was no more common in the patients than in the controls. Furthermore there was no evidence of infection with *Trichomonas vaginalis*, *Candida* species, or viruses, except in isolated cases (Mårdh and Colleen, 1975). In a further series of 21 patients with non-acute prostatitis we could not culture *Staphylococcus saprophyticus* from any of the patients studied using a selective culture technique (Mårdh *et al.*, unpublished data). Thus the results of these microbiological studies, including the present one, suggest that the aetiological agent of non-acute prostatitis is still unknown in most cases.

Negative microbiological findings might suggest a non-infectious cause in many cases of non-acute prostatitis. Patients with this condition often have a poorly developed sex identification and some use their symptoms as an excuse to avoid sexual intercourse, while others repeatedly feel themselves forced to find new female sexual partners to prove their manliness (Nilsson *et al.*, 1975). These latter patients are likely, therefore, to acquire sexually transmitted diseases.

Of the seven patients in the present study with evidence of active chlamydial infection, it is noteworthy that four had previously had gonorrhoea. Of the total of 53 patients, eight had definitely had gonorrhoea. The presence of *C. trachomatis* has been shown to be closely associated with concomitant infection with *N. gonorrhoeae* (Richmond *et al.*, 1972). Of the three remaining patients with seropositive results, one had had acute epididymitis and one had a Reiter-like syndrome. Both these conditions (Harnisch *et al.*, 1977; Keat *et al.*, 1978; Kousa *et al.*, 1978) have been associated with chlamydial infection.

The effect of antibiotic treatment in patients with non-acute prostatitis is difficult to assess, because these patients are often psychologically disturbed (Nilsson *et al.*, 1975). Such disturbances have an effect on symptoms, regardless of whether or not antibiotic treatment is given. Since recurrences are a characteristic of non-acute prostatitis, the length of follow up is also important in assessing the value of any treatment in this condition (Colleen and Mårdh, 1975b).

Tetracycline treatment for one to three weeks in men with acute urethritis from whom *C. trachomatis* had been isolated generally results in negative urethral cultures and a disappearance of the signs

of infection (Oriol *et al.*, 1975; Alani *et al.*, 1977; Ripa *et al.*, 1978a). Although no definite conclusions can be drawn from the seven patients with chlamydial antibodies, it is noteworthy that in those three who were given doxycycline the symptoms and signs had disappeared on re-examination one month after they had completed treatment but that they persisted in the four who received no treatment. Thus, although evidence of *C. trachomatis* infection was not found in many of the patients studied, this does not preclude the fact that this organism may play a role in some cases of non-acute prostatitis.

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