

## DEMONSTRATION OF NEISSERIA GONORRHOEAE IN PROSTATIC FLUID AFTER TREATMENT OF UNCOMPLICATED GONORRHOEAL URETHRITIS

Dan Danielsson and Lars Molin

*From the Department of Clinical Bacteriology, Regional Hospital, Örebro, and the Department of Dermatology, Karolinska sjukhuset, Stockholm, Sweden*

*Abstract.* Prostatic secretion was examined by direct microscopy, fluorescent antibody (FA) techniques and cultural methods in 33 males adequately treated for uncomplicated gonorrhoeal urethritis. The examinations were performed when they were considered cured. In 67% gonococcus-like organisms were observed by direct microscopy, and in 40% a specific identification was arrived at with FA techniques 2 to 3 weeks after treatment. Bacteriological culture was negative. Serological examination indicated an immune response in 78% when the FA test was positive compared with 27% when negative. The importance of the invasion of gonococci into accessory genital glands is discussed.

Figures vary in the literature concerning the involvement of the prostatic gland in association with gonorrhoeal urethritis. Before sulfonamides were introduced the prostate was involved in more than 90% of all male gonorrhoeal infections. Treatment with this drug reduced the figure to about 50%, and during the first years of penicillin therapy the frequency of prostatitis was lowered to about 30% (8). Today prostatitis with clinical symptoms complicating a gonorrhoeal urethritis is considered to be a very uncommon condition. However, Davis has cited the occurrence of prostatitis, as verified by cytology, in 36% of 151 cases with uncomplicated gonorrhoeal urethritis (3).

It is known that a primary gonococcal prostatitis can occur without obvious symptoms of urethritis (7, 9). On these occasions a diagnosis of gonorrhoea can usually be established only by examining exudate obtained by massaging the prostatic gland. On the other hand it is hard to say to what extent the prostate and accessory

genital glands are invaded by gonococci in connection with ordinary gonococcal urethritis where gonococci are demonstrated in urethral secretions. Occasional examination of prostatic secretion after successful treatment of clinically uncomplicated gonococcal urethritis revealed Gram-negative gonococcal-like organisms in the prostatic smears from some of the males. This observation prompted us to undertake a more thorough examination of prostatic secretion at various times after treatment. The results obtained with Gram staining, fluorescent antibody (FA) techniques and cultural methods are presented in the present report. Most of the patients were also examined serologically with the gonococcal complement fixation test.

### MATERIAL AND METHODS

#### *Patients in question*

During a 3 month period every second male attending the out-patient clinic of the Department of Dermatology, Karolinska Hospital, Stockholm, suffering from uncomplicated gonorrhoeal urethritis was studied. The diagnosis of gonorrhoea was established by direct microscopy of smears stained with Gram's technique and/or with methylene blue, and by bacteriological culture according to current techniques. All the isolated gonococcal strains in the present material were highly sensitive to penicillin with a minimum inhibitory concentration less than 0.1 unit of penicillin per ml as determined by the disc method (5).

The patients were treated with penicillin of a dosage recommended by the Royal Swedish Medical Board, i.e. 1.0 megaunit of aqueous benzyl penicillin and 1.5 megaunits of aqueous procain penicillin in a single intramuscular injection. This is standard treatment in Sweden for uncomplicated gonorrhoea in males caused by gonococci

Table I. Results of examinations of prostatic fluid and serum after treatment of uncomplicated gonorrhoeal urethritis

Age	First smear					Second smear			
	Dir. micr. <sup>a</sup>	Cytol. <sup>b</sup>	FA <sup>c</sup>	Bact. culture	Serology	Dir. micr.	Cytol.	FA	Bact. culture
43	++	+	++	0	n. e. <sup>d</sup>	+	-	-	0
21	+	+	++	0	n. e.	+	-	-	0
45	+	+	+	0	1/10	+	+	-	0
22	++	-	++	0	1/80	+	-	-	0
47	+	-	++	0	1/5	+	-	-	0
20	+	-	++	0	1/5	+	-	-	0
26	+	-	++	0	n. e.	?	-	-	0
23	++	-	+	0	1/10	+	-	-	0
19	++	-	+	0	n. e.	+	-	-	0
27	+	-	+	0	-	-	-	-	0
37	+	-	+	0	1/5	-	-	-	0
21	?	-	++	0	-	+	-	-	0
21	-	-	+	0	1/10	-	-	-	0
23	++	++	-	0	-	+	+	-	0
19	+	+	-	0	n. e.	-	-	n. e.	0
23	+	-	-	0	n. e.	-	-	n. e.	0
21	++	-	-	0	1/10	-	-	-	0
35	++	-	-	0	-	-	-	n. e.	0
21	+	-	-	0	n. e.	-	-	n. e.	0
21	+	-	-	0	-	-	-	n. e.	0
22	+	-	-	0	1/10	-	-	n. e.	0
24	+	-	-	0	n. e.	-	-	n. e.	0
26	+	-	-	0	n. e.	-	-	n. e.	0
28	+	-	-	0	n. e.	-	-	n. e.	0
19	?	-	-	0	-	-	-	-	0
22	?	-	-	0	-	-	-	-	0
25	?	-	-	0	-	-	-	-	0
26	-	-	-	0	1/10	-	-	-	0
21	-	-	-	0	n. e.	-	-	-	0
22	-	-	-	0	n. e.	-	-	-	0
22	-	-	-	0	n. e.	-	-	-	0
26	-	-	-	0	-	-	-	-	0
40	-	-	-	0	-	-	-	-	0

<sup>a</sup> ++, several; +, single Gram-negative gonococci-like organisms, either intracellular or extracellular; -, no such organisms.

<sup>b</sup> ++, leucocytes in numerous amount with clumping; +, significant numbers of leucocytes with tendency of clumping; -, no or few leucocytes.

<sup>c</sup> ++, cocci stained by the antigenococcal conjugate with a clearcut morphology; +, with somewhat distorted morphology;

<sup>d</sup> n. e., not examined.

highly sensitive to penicillin. It gives a primary cure rate of 97 to 99% (6, 11).

In all cases of the present report the discharge ceased within 2 to 3 days of the injection of penicillin. The efficacy of the treatment was checked by three weekly examinations including direct microscopy of stained urethral smears and bacteriological culture of urethral specimens.

#### Examination of prostatic fluid

Two to three weeks after treatment prostatic fluid was obtained by digital massage of the prostatic gland immediately after the emptying of the urinary bladder. Specimens were taken for bacteriological culture and smears were prepared on ordinary glass slides, four slides from each patient. Two smears were stained with methylene blue and Gram's technique respectively and scrutinized as regards cytology and the presence of Gram-negative diplococci.

The remaining slides were stained with fluorescein isothiocyanate (FITC) labelled rabbit antigenococcal globulin, diluted 1:20, and mixed with an equal part of undiluted lissamine rhodamine B (RB 200) labelled rabbit anti *S. aureus* globulin. This technique blocks the staining of staphylococci with the antigenococcal conjugate, thus allowing the specific immunofluorescent identification of *Neisseria gonorrhoeae*. At the same time it gives a counterstain of the smears which facilitates the microscopic examination of the slides. The details of this technique are described elsewhere (1). Smears containing cocci, stained by the antigenococcal conjugate and with a clearcut morphology, were graded ++. Smears containing fluorescent cocci with somewhat distorted morphology were graded +, and negative smears -.

No further treatment was given. After another 2 to 3 weeks (i.e. 4 to 6 weeks after treatment) prostatic fluid was again investigated in those cases where the FA test

previously indicated the presence of gonococci in the prostatic fluid, and also in some of those where the FA test was negative but the direct microscopy of the smears previously revealed Gram-negative diplococci.

Smears prepared for the FA technique were coded and examined without information on the history of the patient or the result of the direct microscopy.

#### *Gonococcal complement fixation test*

Blood samples were drawn from 20 patients at the same time as prostatic specimens were taken. Complement fixation tests were carried out according to the technique described by Magnusson & Kjellander (10).

#### *Controls*

As controls 25 males, aged between 18 and 37, without a history of previous urethritis and without symptoms, attending the out-patient clinic because of skin disorders such as acne, mild psoriasis and eczema, were investigated. No one had received antibiotics during the 3 to 4 months preceding the investigation. In all cases the palpation of the prostate was quite normal and urethral specimens examined by direct microscopy of stained smears and by bacteriological cultures were negative.

## RESULTS

The results and findings in the patients examined are presented in Table I.

It will be seen that on the examination of smears prepared from prostatic fluid 2 to 3 weeks after treatment, Gram-negative diplococci were observed in 21 of the 33 patients. Gonococci were identified with FA tests in 11 of these patients, and also in another 2 cases. Bacteriological culture of prostatic secretion gave negative results in all the patients.

On reexamination of the patients 2 to 3 weeks later without any further treatment, all the patients with previously positive FA tests were now regarded negative in the FA test. Gram-negative diplococci were still observed sparsely on smears from 10 of the patients, 9 of whom had positive FA tests earlier. Direct microscopy of smears from 5 of the 10 cases with a negative FA test but previously direct microscopy were judged as negative. No further FA tests were performed in these cases. Bacteriological culture also gave negative results this time.

Serum samples were obtained from 20 patients and were examined for gonococcal antibodies with the complement fixation test. Serum samples from 10 of the patients gave positive results with titres ranging from 1:5 to 1:80. Seven of these patients had positive FA tests for gonococci.

None of the patients had clinical symptoms of prostatitis. The cytology of the smears from 5 of the patients was regarded as positive for prostatitis. Gram-negative diplococci were observed in the prostatic smears from these patients, and gonococci were identified with FA tests in 3 of them.

In the control group no bacterial were found in the direct microscopy of prostatic smears. In five of the cases there were significant numbers of leucocytes with some tendency of clumping, thus judged as pathological (+). In all the cases the FA test was negative.

## DISCUSSION

All the males studied in this report fulfilled the criteria of a successful treatment of an uncomplicated gonorrhoeal urethritis: the absence of symptoms in combination with a negative smear and a negative culture of urethral specimens. However, on examination of prostatic secretion 2 to 3 weeks after treatment, i.e. when the patients were regarded as cured, Gram-negative gonococcal-like organisms were observed in 67%, and by the use of immunofluorescent techniques a specific identification was arrived at in 40% of the patients.

The findings thus indicate that the accessory genital glands are invaded by gonococci in at least 40% of male patients with uncomplicated gonorrhoeal urethritis, and also that the gonococci remain in these glands for up to at least 3 weeks after adequate treatment. It is justified to question the practical importance of these findings, especially the possibility of such a male being a source of infection.

First it should be pointed out that culture of prostatic secretion gave no growth of gonococci in any of the patients examined. This might indicate that the gonococci identified with immunofluorescence were non-viable since the FA method is not dependent upon live organisms. Second, we have no epidemiological data available indicating that the patients presented here could have acted as sources of infection during the observation period. However, since this investigation was completed we have collected data on a group of male patients with repeated negative smears and culture but named as sources of gonorrhoeal infections. These patients had a history of gonorrhoeal and/or "non-gonorrhoeal" urethritis within the pre-

vious 1 to 2 years. Culture of prostatic secretions was positive in only 1 of 7 cases but direct microscopy and FA tests were positive for gonococci. Recently it has been shown by Dunn et al. (4) and by Stamey et al. (13) that prostatic secretion has an antibacterial effect. This could explain the negative yield by culture since it is well known that gonococci are highly fastidious organisms. The epidemiological observations indicate, however, that the "non culturable" gonococci might well be infective when they change their environment.

It is of interest to note that the gonococcal complement fixation test gave positive results in 78% of the patients with positive FA tests after treatment as compared with 27% of those with negative FA tests. The figure of 27% is in accord with serological findings in males with uncomplicated gonorrhoeal urethritis (2, 10, 12). These results suggest that there is an immune response in most of the patients where the accessory genital glands are invaded by gonococci. Whether this immune response contributes to a local immunity remains to be clarified.

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Lars Molin, M.D.  
Department of Dermatology  
Karolinska sjukhuset  
S-104 01 Stockholm 60  
Sweden