Diagnostic Considerations and Interpretation of Microbiological Findings for Evaluation of Chronic Prostatitis

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Seventy-five patients attending a clinic for chronic prostatitis were evaluated by use of lower urinary tract localization cultures. Coagulase-negative staphylococci, alpha-hemolytic streptococci, and diphtheroids were the most common isolates, but none of these organisms were pathogens, based on the absence of bacteriuria or evidence of an inflammatory response in prostatic secretions. Recognized uropathogens were isolated in 12 (16%) of the 75 cases and included *Escherichia coli* in 6 cases, *Enterococcus* spp. in 2 cases, and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Staphylococcus saprophyticus* in 1 case each. Laboratory evaluation of men with chronic prostatitis should concentrate on the isolation and antimicrobial susceptibility testing of bacteria that have an established pathogenic potential in the genitourinary tract.

Prostatitis is a common clinical problem. By one estimate half of all men experience symptoms of prostatitis at some time in their lives (16). These symptoms cause significant morbidity and represent a major indication for diagnostic procedures, treatment with antimicrobial drugs or other medications, or surgical procedures (7, 11, 15).

This paper describes our experience with a standardized protocol for the evaluation of lower urinary tract localization (LUTL) specimens in a carefully defined patient population. Specific goals were to: (i) familiarize clinical microbiologists with techniques used to diagnose prostatitis syndromes, (ii) describe direct Gram stain quantitation of leukocytes in lower urogenital tract specimens, (iii) encourage identification and antimicrobial susceptibility testing of organisms that cause morbidity in men with prostatitis, and (iv) minimize the workup of aerobic bacteria that do not appear to be clinically significant.

MATERIALS AND METHODS

Patient population. All patients who attended a special urology clinic at University Hospital, Seattle, Wash., between 15 June 1984 and 31 December 1987 and who met the clinical criteria for chronic prostatitis were offered a comprehensive evaluation of their problem (including microbiological and structural causes). Of more than 100 patients seen, 75 elected to have the diagnostic evaluation described in this study. The two most common reasons why patients declined to undergo the evaluation were that they desired immediate treatment or that they wished to avoid uncomfortable procedures.

The mean age of the patients in this study was 39.6 years (median, 38 years; range, 15 to 72 years). Seventy of the patients (93%) were heterosexual, and five (7%) were homo- or bisexual. Thirty patients (40%) were married.

Definitions. A standard set of definitions was applied by the single urologist who evaluated all the patients.

(i) **Chronic prostatitis.** Patients were considered to have chronic prostatitis if they met three criteria: (i) the patient attended the specialized clinic for prostatitis, (ii) symptoms had persisted for at least 3 months, and (iii) the patient had been told that he had prostatitis and had been treated

previously by at least one physician. Patients with chronic prostatitis syndromes were further classified into three groups, chronic bacterial prostatitis, nonbacterial prostatitis, and prostatodynia, following the criteria described by Stamey and others (2, 11, 16). Chronic bacterial prostatitis was defined as documented episodes of bacteriuria caused by the same bacterial species that were localized in the prostate. Nonbacterial prostatitis was defined as objective evidence of prostatic inflammation but no evidence of bacterial prostatitis. Prostatodynia was defined as symptoms but no evidence of either bacterial prostatitis or prostatic inflammation.

(ii) **Inflammation.** Inflammation was defined as ≥ 12 leukocytes per high-power field (hpf) ($\times 400$) of Gram-stained prostatic secretions (1, 16).

(iii) **Prostatic focus.** A bacterial species was considered to be localized in the prostate if there was at least a 10-fold increase in CFU per milliliter between the first-void urine (VB1) specimen and the postmassage urine (VB3) specimen or the expressed prostatic secretions (EPS) if the VB3 specimen did not meet this criterion (11, 12, 16).

Clinical evaluation. At the initial visit a standardized history was taken and a general medical examination was performed, with specific attention to the urogenital organs. Patients in the study had received no antimicrobial agents or other drugs for the management of prostatitis for at least 2 weeks. They were seen in the prostatitis clinic in the morning prior to the initial micturition of the day. They were instructed not to ejaculate for at least 2 days prior to the prostatic evaluation. The protocol focused on detecting the presence of leukocytes and bacteria in the urine and prostatic secretions, both by Gram staining and culturing, as well as detecting significant structural or functional abnormalities of the lower urogenital tract. A complete description of the urological findings is the subject of another communication (J. N. Krieger and K. J. Egan, manuscript in preparation).

LUTL specimen collection. LUTL specimen collection was originally described by Meares and Stamey (12). Several technical points deserve emphasis. The foreskin of uncircumcised patients was retracted and taped in place. The glans was cleaned with sterile water and dried prior to obtaining the first specimen. The use of water for cleansing is important because antimicrobial preparations may lead to

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artificial reductions in colony counts or a false-negative culture (16). After the VB1 specimen and the midstream (bladder) urine (VB2) specimen were obtained, the patient was instructed to stop voiding, and any residual urine was stripped from the urethra. EPS were obtained by massage, followed by the VB3 specimen. The specimens were transported immediately to the microbiology laboratory.

Bacteriological materials and methods. (i) Gram stain evaluation. VB1, VB2, EPS, and VB3 specimens were examined by direct Gram staining and quantitative culturing in the microbiology laboratory. The urine Gram stain was performed by placing 30 μ l of well-mixed unspun urine on a precleaned glass slide, between two lines etched on the slide approximately 15 mm apart, and air drying. The EPS Gram stain was performed in the same way with 1 drop of undiluted EPS. The Gram-stained smears were scanned at $\times 400$ and areas with maximal concentrations of cells and/or organisms were quantified under oil immersion ($\times 1,000$). The presence of any cells and/or organisms was reported.

(ii) Cultures and growth conditions. The urine was quantitatively cultured with 0.1-ml samples of urine each delivered by a micropipette to one entire heart infusion agar plate containing 5% defibrinated sheep blood (Difco Laboratories, Detroit, Mich.) and one entire MacConkey agar plate (without crystal violet) (10). The EPS were cultured in an identical fashion, unless there was <0.1 ml, in which case 0.01-ml samples each were delivered by a micropipette to one entire blood agar plate and one entire MacConkey agar plate. The cultures were incubated at 35°C in an atmosphere of 10% CO₂ and examined after overnight incubation and again after an additional 18 to 24 h of incubation.

(iii) Identification and susceptibility testing. Colonies were Gram stained and biochemical properties were determined by standard methods (9), by the AutoMicrobic system GNI card (Vitek Systems, Inc., Hazelwood, Mo.), and by the API 20E and Staph-Ident systems (Analytab Products, Plainview, N.Y.). Antimicrobial susceptibility was determined by the standardized disk diffusion method (13).

Clinical results. Patients were reevaluated clinically for a minimum of 3 months, and repeat LUTL studies were done for those with microbiological findings as well as for patients with persistent symptoms.

RESULTS

Laboratory findings. Inflammation. The mean number of leukocytes in EPS was 6 per hpf (median, 0; range, 0 to 100). Of the 75 patients, 60 (80%) had no evidence of prostatic inflammation and 15 had ≥ 12 leukocytes per hpf of EPS. Patients with chronic bacterial prostatitis had a mean of 34 leukocytes per hpf (median, 25; range, 0 to 100) of EPS, patients with nonbacterial prostatitis had a mean of 21 leukocytes per hpf (median, 17.5; range, 12 to 50) of EPS, and patients with prostatodynia had a mean of 1 leukocyte per hpf (median, 0; range, 0 to 10) of EPS.

Microbiology. (i) Quantitative results. Although there was considerable variation, most patients had fewer than 1,000

TABLE 1. Total aerobic bacterial counts in LUTL specimens

Specimen	Mean CFU/ml	SD	Median CFU/ml	Range of CFU/ml
VB1	11,118	34,650	800	<30–210,920
VB2	7,693	25,736	80	<30–100,070
EPS	6,699	21,456	500	<30–103,000
VB3	6,643	23,370	130	<30–100,140

TABLE 2. Number of species isolated in LUTL cultures for 75 men with chronic prostatitis

Specimen	No. of men from whom the following no. of species was isolated:					
	0	1	2	3	4	5
VB1	15	12	26	16	4	2
VB2	26	17	23	6	3	0
EPS	24	21	14	14	2	0
VB3	0	41	19	11	4	0

CFU/ml in all of their LUTL cultures (Table 1). The median counts were 800 CFU/ml for VB1, 80 CFU/ml for VB2, 500 CFU/ml for EPS, and 130 CFU/ml for VB3.

The numbers of species isolated in aerobic LUTL cultures for the 75 patients with chronic prostatitis are summarized in Table 2. Bacteria were isolated from VB1 in 60 cases (80%), from VB2 in 49 cases (65%), from EPS in 51 cases (68%), and from VB3 in all 75 cases. The median numbers of species isolated were two in VB1 and one each in VB2, EPS, and VB3. Coagulase-negative staphylococci, alpha-hemolytic streptococci, and diphtheroids were the most common isolates in all lower urinary tract specimens (Table 3).

(ii) Organisms isolated from the prostate. Thirteen patients had prostatic localization of organisms; seven of these patients had recognized uropathogens and met the criteria for chronic bacterial prostatitis (Table 4). Each of the seven had documented bacteriuria caused by the same organism that was localized in the prostate in subsequent LUTL studies. Organisms isolated included *Escherichia coli* in five cases and *Pseudomonas aeruginosa* and *Enterobacter cloacae* in one case each. Six patients had LUTL study results consistent with prostatic localization of organisms but without documented bacteriuria. The organisms isolated from this group have no proven pathogenic potential in the male genitourinary tract and included beta-hemolytic streptococci in two cases and alpha-hemolytic streptococci, coagulase-negative staphylococci (not *Staphylococcus saprophyticus*), lactobacilli, and diphtheroids in one case each (Table 4). These organisms were isolated from five patients with prostatodynia and one patient with nonbacterial prostatitis.

Five additional patients had documented bacteriuria ($>10,000$ CFU/ml of VB2), but the organisms were not localized, so these patients did not meet the microbiological criteria for bacterial prostatitis in subsequent LUTL studies. The organisms isolated from these men included *Enterococcus* spp. in two cases and *E. coli*, *Klebsiella pneumoniae*, and *S. saprophyticus* in one case each.

TABLE 3. Bacteriological findings in 75 men with chronic prostatitis

Organism	No. of the following specimens with the indicated organism:			
	VB1	VB2	EPS	VB3
Coagulase-negative staphylococci	52	32	38	42
Alpha-hemolytic streptococci	28	22	18	24
Diphtheroids	28	16	15	16
<i>Enterococcus</i> spp.	11	8	9	7
Group G streptococci	6	7	5	6
Lactobacilli	5	4	5	5
<i>E. coli</i>	4	3	6	4
Beta-hemolytic streptococci	3	1	2	2
<i>E. cloacae</i>			1	
None (no growth)	15	26	24	22

TABLE 4. Cultures consistent with prostatic localization of bacteria

Patient	CFU/ml in:				Organism	Bacteriuria ^a	EPS inflammatory response (no. of leukocytes per hpf)
	VB1	VB2	EPS	VB3			
1	— ^b	—	1,500	10	<i>E. cloacae</i>	+	0
2	1,200	1,200	15,000	4,400	<i>E. coli</i>	+	30
3	—	—	4,000	110	<i>E. coli</i>	+	100
4	100	200	2,700	110	<i>E. coli</i>	+	20
5	—	—	50,000	300	<i>P. aeruginosa</i>	+	100
6	300	240	2,400	270	<i>E. coli</i>	+	50
7	—	—	100	—	<i>E. coli</i>	+	5
8	—	—	14	—	Beta-hemolytic streptococci	—	20
9	1,500	1,000	100,000	290	Coagulase-negative staphylococci ^c	—	0
10	270	—	3,200	240	Lactobacilli	—	1
11	10	—	300	—	Beta-hemolytic streptococci	—	0
12	30	30	800	30	Alpha-hemolytic streptococci	—	0
13	120	20	2,000	240	Diphtheroids	—	0

^a Presence (+) or absence (—) of previous bacteriuria caused by the species that was subsequently localized in the prostate.

^b —, Fewer than 10 CFU of the organism localized in the prostate per ml.

^c Not *S. saprophyticus*.

(iii) **Inflammatory response.** The seven patients with chronic bacterial prostatitis caused by recognized uropathogens had a mean of 44 leukocytes per hpf (median, 30; range, 0 to 100) of EPS. In contrast, the six patients who had other bacteria localized in the prostate but who had no history of bacteriuria had a mean of 4 leukocytes per hpf (median, 0; range, 0 to 20) of EPS ($P = 0.0426$; two-tailed t test).

Clinical classification of patients, treatment, and outcome. Of the 75 patients with chronic prostatitis, 7 (9%) had chronic bacterial prostatitis, 8 (11%) had nonbacterial prostatitis, and 60 (80%) had prostatodynia. The seven patients with chronic bacterial prostatitis received 3 months of treatment with trimethoprim-sulfamethoxazole. Five were cured of infection, as determined by at least two subsequent localization studies after the completion of treatment. The other two patients remain asymptomatic on continuous, low dosages of antimicrobial drugs despite persistent prostatic foci of infection. Each of the five patients who had documented bacteriuria and who did not meet the criteria for bacterial prostatitis received a 7- to 10-day course of antimicrobial drugs, such as nitrofurantoin, that have poor penetration of uninflamed prostatic parenchyma. All responded well and had at least two negative localization studies following the completion of therapy.

The six patients with other organisms localized in the prostate but with no evidence of bacteriuria received no treatment. Follow-up of three of these patients after 3 months showed that two estimated that their symptoms were at least 90% resolved. The remaining patient experienced transient improvement after discontinuing previous antimicrobial therapy, followed by a recurrence.

Of the 75 patients, 14 had other organisms isolated (*Chlamydia trachomatis*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, and *Mycoplasma hominis*) and 8 had significant structural or functional abnormalities (Krieger and Egan, in preparation). There was no statistically significant difference in the prevalence of these organisms among the three patient groups.

DISCUSSION

Microbiological criteria for the classification of prostatitis syndromes were suggested by Meares and Stamey and others (2, 12). These investigators proposed that patients with bacteriuria should be distinguished from those with no

evidence of bacteriuria and that careful LUTL studies could be used to differentiate four syndromes: acute bacterial prostatitis, chronic bacterial prostatitis, nonbacterial prostatitis, and prostatodynia. Patients with bacterial prostatitis have (i) urinary tract infections caused by recognized uropathogens, (ii) objective evidence of prostatic inflammation, and (iii) a prostatic focus of infection.

Patients with acute bacterial prostatitis seldom pose major diagnostic or therapeutic problems (11, 16). Acute bacterial prostatitis is characterized by the isolation of high colony counts of recognized uropathogens in the midstream urine from patients with a characteristic clinical syndrome. Clinical findings that suggest acute bacterial prostatitis include lower urinary tract irritative symptoms, such as urinary urgency and frequency, systemic signs and symptoms, and an abnormally tense prostate (7, 11, 16). The causes and potentially life-threatening consequences of acute bacterial prostatitis are usually apparent, and patients respond dramatically to appropriate antimicrobial therapy.

In contrast to acute bacterial prostatitis, chronic prostatitis syndromes challenge both the clinician's diagnostic acumen and the resources of the clinical microbiology laboratory. These syndromes include chronic bacterial prostatitis, nonbacterial prostatitis, and prostatodynia. Chronic bacterial prostatitis is characterized by recurrent urinary tract infections caused by the same bacterial species. Patients are often asymptomatic between episodes of bacteriuria, seldom have abnormalities on physical examination, and require prolonged treatment with antimicrobial drugs. During the last 2 decades there have been many valuable studies of chronic bacterial prostatitis. These efforts have improved our understanding of both specific immunological factors and nonspecific host defenses of the male lower urogenital tract, such as pH and the prostatic antibacterial factor (3, 4, 6, 8, 14, 17).

Of the 75 patients with chronic prostatitis in this study, 7 (9%) met the criteria for chronic bacterial prostatitis. Organisms isolated from these seven patients included *E. coli* in five cases and *P. aeruginosa* and *E. cloacae* in one case each. However, five other patients also had episodes of well-documented bacteriuria caused by recognized uropathogens that could not be localized in the prostate in repeated studies. Organisms isolated from these patients included *Enterococcus* spp. in two cases and *E. coli*, *K.*

pneumoniae, and *S. saprophyticus* in one case each. These findings suggest that the standard definition of a 10-fold increase in the CFU of recognized uropathogens per milliliter may be specific for the diagnosis of chronic bacterial prostatitis in research studies but may lack sensitivity in routine clinical practice. Of the 12 patients with known uropathogens (7 with organisms localized in the prostate and 5 with documented bacteriuria only), all had good clinical outcomes following appropriate antimicrobial therapy.

Most patients with chronic prostatitis have nonbacterial prostatitis or prostatodynia (7, 16). The distinction between nonbacterial prostatitis and prostatodynia is based on objective evidence of an inflammatory response in prostatic secretions of patients with nonbacterial prostatitis and the absence of objective evidence of an inflammatory response in prostatic secretions of patients with prostatodynia. Of the 75 patients with chronic prostatitis in this study, 8 (11%) had nonbacterial prostatitis and 60 (80%) had prostatodynia. The causes and natural history of these conditions are poorly defined, and optimal treatment is uncertain.

The isolation of organisms with no clear pathogenic potential from the lower urinary tract represents a possible source of confusion for microbiologists and clinicians. Several findings support the conclusion that such organisms are nonpathogens that have no role in the etiology of chronic prostatitis. First, no patient had bacteriuria caused by alpha- or beta-hemolytic streptococci, coagulase-negative staphylococci (not *S. saprophyticus*), lactobacilli, or diphtheroids, despite the apparent prostatic localization of these organisms. Second, patients with these organisms had significantly less of an EPS inflammatory response than did patients with chronic bacterial prostatitis. The seven patients with chronic bacterial prostatitis caused by recognized uropathogens had a mean of 44 leukocytes per hpf of EPS, while the six patients with other bacteria localized in the prostate but with no history of bacteriuria had a mean of 4 leukocytes per hpf of EPS ($P < 0.05$). Third, prior to evaluation all patients in this series had received multiple courses of antimicrobial drugs, often directed against such organisms, with no success. A similar spectrum of nonpathogenic bacteria was isolated from urethral urine (VB1) cultures for the overall population of patients in this study (Table 3). A higher proportion of apparent prostatic localization would have been obtained if less attention had been directed to the clinical technique. For example, if microbical preparations had been used to clean the meatus, the VB1 counts would have been reduced, incorrectly suggesting many prostatic foci of nonpathogens. This conclusion agrees with that of Fowler and Mariano, who performed serial localization cultures for six healthy men (5). The cultures often suggested prostatic infection by organisms such as coagulase-negative staphylococci or diphtheroids, suggesting that EPS were more susceptible to contamination by urethral bacteria than was urine, owing to greater viscosity and smaller volume.

An accurate diagnosis of patients with prostatitis symptoms is important as a guide for treatment. Appropriate antimicrobial therapy is effective for patients with bacterial prostatitis. Acute bacterial prostatitis usually responds completely to a single course of therapy (16). Chronic bacterial prostatitis requires prolonged treatment: many patients are cured following 3 months of full-dose treatment with drugs that penetrate the prostatic parenchyma, while patients who are not cured are rendered asymptomatic by continuous, low-dose antimicrobial treatment. In contrast, patients with

nonbacterial prostatitis and prostatodynia derive little benefit from antimicrobial therapy (7, 16).

This study has three important implications for clinical microbiology laboratories. First, clear communication between physicians and laboratories is essential to ensure that optimal specimens are obtained, transported expeditiously, and appropriately handled in the laboratory. Laboratory personnel should understand the nature of the LUTL specimens and the clinical problem. LUTL procedures are uncomfortable, expensive, and time-consuming. Second, efforts should be directed toward the isolation and antimicrobial susceptibility testing of bacteria that have established pathogenic potential in the genitourinary tract. These organisms include members of the family *Enterobacteriaceae*, *Enterococcus* spp., *P. aeruginosa*, and *S. saprophyticus*. Antimicrobial therapy is optimally used to eliminate such uropathogens from men with bacterial prostatitis or documented bacteriuria. The total CFU per milliliter are much less important than the CFU of recognized uropathogens per milliliter (Table 4). Third, determination of the species of organisms, such as coagulase-negative staphylococci other than *S. saprophyticus*, alpha-hemolytic streptococci, diphtheroids, and similar bacteria, isolated from the male lower urinary tract probably represents a poor use of laboratory resources. The information is seldom useful for patient management and may be misleading. Antimicrobial susceptibility testing of such organisms may be interpreted as an indication for the treatment of organisms with minimal pathogenic potential. Our experience is that patients with symptoms of chronic prostatitis receive many unsuccessful courses of antimicrobial drugs (7, 16). Optimal use of clinical laboratory resources can be achieved by emphasizing identification and antibiotic susceptibility testing of recognized uropathogens.

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LITERATURE CITED

1. Anderson, R. U., and C. Weller. 1979. Prostatic secretion leukocyte patterns in nonbacterial prostatitis (prostatosis). *J. Urol.* 121:292-294.
2. Drach, G. W., E. M. Meares, Jr., W. R. Fair, and T. A. Stamey. 1978. Classification of benign disease associated with prostatic pain: prostatitis or prostatodynia? *J. Urol.* 120:266.
3. Fair, W. R., J. Couch, and N. Wehner. 1976. Prostatic antibacterial factor. Identity and significance. *Urology* 7:169-177.
4. Fowler, J. E., Jr., D. L. Kaiser, and M. Mariano. 1982. Immunologic response of the prostate to bacteriuria and bacterial prostatitis. I. Immunoglobulin concentrations in prostatic fluid. *J. Urol.* 128:158-164.
5. Fowler, J. E., Jr., and M. Mariano. 1984. Difficulties in quantitating the contribution of urethral bacteria to prostatic fluid and seminal fluid cultures. *J. Urol.* 132:471-473.
6. Hanna, L., H. Keshishyan, G. F. Brooks, D. P. Stites, and E. Jawetz. 1981. Effect of seminal plasma on *Chlamydia trachomatis* LB-1 in cell culture. *Infect. Immun.* 32:404-406.
7. Krieger, J. N. 1984. Prostatitis syndromes: pathophysiology, differential diagnosis and treatment. *Sex. Transm. Dis.* 11:100-112.
8. Krieger, J. N., and M. F. Rein. 1982. Canine prostatic secretions kill *Trichomonas vaginalis*. *Infect. Immun.* 37:77-81.

9. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
10. **Mackie, T. J., and J. E. McCartney.** 1956. Handbook of practical bacteriology, p. 180. The Williams & Wilkins Co., Baltimore.
11. **Meares, E. M., Jr.** 1980. Prostatitis syndromes: new perspectives above old woes. *J. Urol.* **123**:141-147.
12. **Meares, E. M., Jr., and T. A. Stamey.** 1968. Bacteriologic localization patterns in bacterial prostatitis and urethritis. *Invest. Urol.* **5**:492-518.
13. **National Committee for Clinical Laboratory Standards.** 1984. Approved standard M2-A3. Performance standards for antimicrobial disc susceptibility tests. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Shortliffe, L. M. D., N. Wehner, and T. A. Stamey.** 1981. Use of solid-phase radioimmunoassay and Formalin-fixed whole bacterial antigen in the detection of antigen-specific immunoglobulin in prostatic fluid. *J. Clin. Invest.* **67**:790-799.
15. **Smart, C. J., and J. D. Jenkins.** 1973. The role of transurethral prostatectomy in chronic prostatitis. *Br. J. Urol.* **45**:654-662.
16. **Stamey, T. A.** 1980. Pathogenesis and treatment of urinary tract infections, p. 212-223. The Williams & Wilkins Co., Baltimore.
17. **Stamey, T. A., W. R. Fair, M. M. Timothy, and H. K. Chung.** 1968. Antibacterial nature of prostatic fluid. *Nature (London)* **218**:444-447.