

Seminal Fluid Findings in Men With Nonbacterial Prostatitis and Prostatodynia

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ABSTRACT: There is considerable confusion about the effects of prostatitis syndromes on male reproductive physiology. Therefore, we correlated findings on seminal fluid and expressed prostatic secretions from 100 men attending a special prostatitis clinic. These men had symptoms of prostatitis but no evidence of urethritis, acute or chronic bacterial prostatitis, or significant urological abnormalities. All subjects were evaluated following a standardized protocol, including lower urinary tract localization studies, expressed prostatic secretion analyses, and seminal fluid analyses with Bryan-Leishman staining. Seminal fluid findings were compared in men with inflammation ($\geq 10^6$ leukocytes/ml) in their expressed prostatic secretions, i.e., nonbacterial prostatitis, and men without inflammation in prostatic secretions, i.e., prostatodynia. Of 23 men with inflammation ($\geq 10^6$ leukocytes/ml) in their seminal fluid, 6 (26%) had nonbacterial prostatitis (mean leukocyte concentration $8.6 \pm 9.4 \times 10^6$ /ml of semen) and 17 (74%) had prostatodynia (mean leukocyte concentration $6.2 \pm 7.0 \times 10^6$ /ml, not significant). Of 77 men who did not have seminal inflammation, 15 (19%) had nonbacterial prostatitis (mean

leukocyte concentration $0.1 \pm 0.2 \times 10^6$ /ml) and 62 (81%) had prostatodynia (mean leukocyte concentration $0.1 \pm 0.2 \times 10^6$ /ml, not significant). Men with nonbacterial prostatitis had lower values for several parameters associated with sperm motility, especially the proportion of motile sperm (45% compared with 60% for men with prostatodynia, $P = 0.08$) and sperm subjective speed score (median 3 compared to 4 for men with prostatodynia, $P = 0.03$). In summary, a minority of men had seminal inflammation, even among men with nonbacterial prostatitis. There was poor correlation between inflammation in the prostatic secretions and in the semen. Nonbacterial prostatitis, but not seminal inflammation, was associated with reduced sperm motility. Our findings highlight technical issues and the importance of investigating different sites and samples, including the urethra, expressed prostatic secretions, and seminal fluid.

Key words: Nonbacterial prostatitis, prostatodynia, seminal inflammation, expressed prostatic secretions, semen analysis, leukocytospermia.

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There is considerable confusion about the clinical findings associated with prostatitis and the effect of these conditions on male reproductive physiology. Much of the confusion reflects differing definitions and clinical approaches to these syndromes in urology and male fertility.

The urologic literature considers patient symptoms, presence or absence of bacteriuria, and inflammation in the expressed prostatic secretions (Stamey, 1980; Krieger and McGonagle, 1989). Based on these findings, symptomatic men are classified in four groups: acute bacterial prostatitis, chronic bacterial prostatitis, nonbacterial prostatitis, and prostatodynia. Presence of bacteriuria is the sine qua non for diagnosis of bacterial prostatitis. Acute bacterial prostatitis is associated with acute lower urinary tract symptoms, bacteriuria, and, often, with signs and symptoms of a systemic illness. The prostate is "hot" and exquisitely tender on physical examination. Chronic bac-

terial prostatitis is associated with recurrent episodes of bacteriuria caused by the same organism. Expressed prostatic secretions are purulent in acute and chronic bacterial prostatitis, reflecting infection of the prostate gland. Although the great majority of the urological literature concerns men with bacterial prostatitis, such patients represent $\leq 10\%$ of the overall population of men with prostatitis syndromes (Krieger and Egan, 1991; de la Rosette et al, 1993).

Most men with symptomatic prostatitis do not have bacteriuria. These men have two other chronic prostatitis syndromes—nonbacterial prostatitis and prostatodynia (Krieger and McGonagle, 1989). Men with nonbacterial prostatitis have symptoms and inflammation in their prostatic secretions, but they do not have either bacteriuria or a bacterial pathogen that repeatedly "localizes" to the prostate on segmented lower urinary tract cultures. In contrast, men with prostatodynia have symptoms but have normal expressed prostatic secretions. The urological approach to prostatitis has at least two major limitations (Krieger and Egan, 1991). First, therapy is unsatisfactory for most men with prostatitis syndromes because we have limited insight into the causes of the most common syndromes. Second, findings in expressed prostatic secretions

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of men with nonbacterial prostatitis and prostatodynia have not been shown to correlate with symptoms, response to treatment, or other physiological parameters.

In the male fertility literature, diagnosis of "prostatitis," "prostato-seminal vesiculitis," "male accessory gland infection," or "epididymo-prostato-vesiculitis," depends on seminal fluid analysis (El Bayoumi et al, 1982; Comhaire et al, 1986; Anderson, 1990; Christiansen et al, 1991; Aitken et al, 1994; Branigan and Muller, 1994). Men with $\geq 10^6$ leukocytes/ml of semen, or >6 leukocytes per 100 sperm, are usually considered to have "leukocytospermia" (also known as "pyospermia"), a finding that suggests prostatic or lower genital tract inflammation. There are major problems with this approach to prostatitis. Investigators have seldom described the symptoms or physical findings in their subjects. Although leukocytes in semen may come from many sites in the lower urogenital tract, investigators have seldom evaluated other sites, such as the urethra, for signs or symptoms of inflammation. Finally, the potential relationship between seminal inflammation and infection is incompletely defined (Purvis and Christiansen, 1993).

The purpose of this study was to correlate findings in seminal fluid and expressed prostatic secretions from a selected population of men attending our prostatitis clinic. Subjects were evaluated according to a standardized protocol, including evaluations to ensure that they did not have urethral inflammation or infection with common urogenital pathogens. By comparing the results of urologic and male fertility approaches, we hoped to gain insights in the pathophysiology of these conditions and to define new directions for future research. Our hypothesis was that men with objective evidence of inflammation in their expressed prostatic secretions, i.e., nonbacterial prostatitis, would be more likely to have seminal inflammation than men who had uninflamed prostatic secretions, i.e., those with prostatodynia.

Materials and Methods

Patients

Subjects were recruited from men attending the Prostatitis Clinic at the University of Washington Medical Center. Study subjects were over 18 years old, spoke English, and were able to provide informed consent. Potential subjects were offered a standardized evaluation for infectious, inflammatory, structural, and functional conditions associated with prostatitis. The protocol was approved by the University of Washington Human Subjects Committee.

Definitions and Selection Criteria

We used classification schemes employed in both urology and in male fertility. The urologic literature concentrates on evaluation of men with lower genitourinary tract symptoms using seg-

mented specimens obtained in a standardized fashion (Stamey, 1980; Krieger and McGonagle, 1989). These specimens include first-void urine, mid-stream urine, expressed prostatic secretions, and post-massage urine samples, as described below. A uropathogenic organism "localizes" to the prostate when there is a ten-fold increase in concentration from the first void urine to the post-massage urine or the expressed prostatic secretion sample.

Men with acute and chronic bacterial prostatitis have bacteriuria caused by the same uropathogen that localizes to their prostates, and they have inflamed prostatic secretions (Anderson and Weller, 1979; Stamey, 1980). Men with bacterial prostatitis, acute or chronic, were excluded from this study. This study was limited to the great majority of men with prostatitis who did not have bacteriuria, including nonbacterial prostatitis and prostatodynia. Men with nonbacterial prostatitis have inflammation in their prostatic secretions whereas men with prostatodynia do not.

The male fertility literature concentrates on seminal fluid analysis. Various terms include "leukocytospermia" (Wolff et al, 1990; Gonzales et al, 1992; Branigan and Muller, 1994; Yanushpolsky et al, 1995), "prostato-seminal vesiculitis" (El Bayoumi et al, 1982; Comhaire et al, 1986; Christiansen et al, 1991; Purvis and Christiansen, 1993), and "male accessory gland infection" (Comhaire et al, 1986; Purvis and Christiansen, 1993). To limit the potential for confusion, our preferred usage was "seminal inflammation." Subjects were considered to have seminal inflammation if they had $\geq 10^6$ leukocytes/ml of semen.

Clinical and Microbiological Methods

This study was limited to men with chronic prostatitis, defined as symptoms for at least 3 months (Krieger and Egan, 1991). All studies were done while the subject was off all antimicrobial and anti-inflammatory therapy for at least 4 weeks. Patients were evaluated during a series of three visits.

During the first visit the patient had a standardized history and physical examination, including review of previous cultures and treatment. Men who had had previous prostate surgery were excluded from the study. Urethral samples were obtained for gram-stained urethral smear and cultures for *Neisseria gonorrhoeae* using modified Thayer-Martin medium (Bowie et al, 1977), *Chlamydia trachomatis* using cyclohexamide-treated McCoy cells (Stamm et al, 1983), *Trichomonas vaginalis* using modified Diamond's medium (Krieger et al, 1988b), and genital mycoplasmas using A7 agar plates and broth medium (Kenny, 1991). Potential subjects were excluded from the study if they had urethritis (≥ 5 leukocytes/400 \times microscopic field of the urethral smear) or a positive urethral culture for *N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*. Men with *Mycoplasma hominis* or *Ureaplasma urealyticum* were included in the study if their symptoms persisted after completion of antimicrobial treatment.

At least 2 weeks later, the patient was instructed to return with a full bladder and not having ejaculated for at least 3 days. At this visit, each patient was reevaluated clinically and had a lower urinary tract localization study, following established methods (Krieger and McGonagle, 1989). Leukocytes in expressed prostatic secretions were enumerated by hemocytometer counts. The patient was asked to provide a semen sample at this visit, if possible. Patients who could not provide a semen sample in the clinic were asked to return with a fresh (<2 hours old) specimen.

At the third visit, the patient had a urinary flow study and determination of post-void residual urine using a dedicated ultrasound instrument (Krieger et al, 1988a). Patients with objective evidence of lower urinary tract obstruction or functional abnormalities underwent appropriate urological evaluation. Men with clinically significant structural abnormalities, i.e., small caliber urethral strictures, were excluded from the study.

Semen Processing, Staining, and Analysis

Samples were collected by masturbation into sterile containers (polypropylene, nontoxic to sperm) after 3–7 days of sexual abstinence and examined within 2 hours. All semen analyses were performed in the Male Fertility Laboratory in the University of Washington Department of Urology. Analysis time, liquefaction, viscosity, color, volume, and pH were recorded. A wet drop of 5–6 μ l was examined using phase-contrast microscopy for progressive motility, rapid and linear motility, subjective speed and progress, "round cells" per high-power field (hpf), and sperm agglutination, by counting at least 100 sperm or 25 fields. If motility was <25%, a live–dead stain was performed. Concentration was measured using immobilized sperm in a hemocytometer. During the latter portion of the study, computerized strict criteria morphology on Papanicolaou-stained thin smears was performed using the Hamilton Thorne Research (Beverly, Massachusetts) "Dimensions" program. For each semen analysis, a thin smear was stained using modified Bryan-Leishman stain and examined for sperm and leukocyte morphology according to the World Health Organization (WHO) protocol (Couture et al, 1976; World Health Organization, 1992). This method allows discrimination of leukocytes from immature sperm forms. Leukocyte types were enumerated per 100 sperm. Then the concentrations (millions/ml) of total leukocytes and each leukocyte subset (polymorphonuclear cells, lymphocytes, and monocytes) were calculated by multiplying the leukocytes per 100 sperm times the sperm concentration and dividing by 100. We found excellent agreement between this staining and evaluation of the same samples by monoclonal antibody HLe-1 (panleukocyte)-stained slides (Branigan and Muller, 1994). Weekly quality control was provided by cryopreserved aliquots of semen or by semen samples from normal donors.

Statistical Analysis

The Mann-Whitney *U*-test was used to compare continuous measurements for different groups of subjects. The chi-square test was used to compare data in contingency tables, where the data in the rows and columns represented independent observations. McNemar's test was used to evaluate data in contingency tables if the rows and columns were not independent.

Results

Characteristics of the Study Population

We evaluated a total of 231 men who did not have acute or chronic bacterial prostatitis. Of these 231 men, 164 (71%) had lower urinary tract localization studies, and 133 (51%) provided semen samples. The median time

until semen analysis was 35 minutes (range 15–120 minutes), and the time interval had no significant effect on the proportion of abnormal findings (data not shown). One hundred patients who did not have urethritis or significant lower urinary tract obstruction provided both lower urinary tract localization studies and semen samples, representing 34% of the 231 men in the overall population. The median interval between the semen analysis and evaluation of the expressed prostatic secretions was 0 days (mean \pm 1 standard deviation [SD] 3.2 ± 9.0 days). The median interval was 0 days for men with nonbacterial prostatitis (mean 1.0 ± 3.2 days) and 0 days (mean 2.9 ± 7.9 days) for men with prostatodynia (not significant). The median interval was also 0 days for men with or without seminal inflammation (not significant).

The mean age (\pm SD) for the overall population was 38.0 ± 12.1 years (range 18–77), compared to 35.7 ± 11.0 years for the men who had lower tract localization studies, 38.0 ± 12.5 years for men who provided semen samples, and 35.9 ± 11.4 years for subjects who provided both localization studies and a semen specimen. None of the age differences was significant. Of the 231 men in the overall population, 197 (85%) were white, 15 (6.5%) were black, 10 (4.3%) were Asian or Pacific islanders, 5 (2.2%) were Hispanic, 1 (0.4%) was a Native American, and 4 (1.7%) were members of other groups. Of the 100 men who provided both lower urinary tract localization and semen samples, 81 were white, 7 were black, 7 were Asian or Pacific islanders, 2 were Hispanic, 1 was Native American, and 2 were members of other groups (not significantly different from the overall population). These distributions were similar to the overall racial distribution of men attending the Urology Clinic at University of Washington Medical Center.

Seminal Fluid Inflammation Compared With Chronic Prostatitis Syndromes

Of the 100 men who had both semen analyses and examination of expressed prostatic secretions, 21 had nonbacterial prostatitis and 79 had prostatodynia. The median concentration of leukocytes was 2.5×10^6 /ml (range 1.0 – 36.0×10^6 /ml) in the expressed prostatic secretions of men with nonbacterial prostatitis, including a median of 1.2×10^6 polymorphonuclear cells (range 0 – 10.0×10^6 /ml) and a median of 1.0×10^6 /ml other leukocytes (range 0 – 28.9×10^6 /ml). The median concentration of leukocytes was 0.1×10^6 /ml (range 0 – 0.9×10^6 /ml) in the expressed prostatic secretions of men with prostatodynia, including a median of 0.1×10^6 polymorphonuclear cells (range 0 – 0.9×10^6 /ml) and a median of 0 /ml other leukocytes (range 0 – 0.4×10^6 /ml).

Mean concentrations of total seminal leukocytes, seminal polymorphonuclear leukocytes, and other leukocytes were all higher in men with nonbacterial prostatitis than

Table 1. Seminal fluid leukocyte concentrations in 100 men with chronic nonbacterial prostatitis and prostatodynia

Seminal fluid leukocytes ($\times 10^6/\text{ml}$)	Population		Significance*
	Nonbacterial prostatitis (N = 21)	Prostatodynia (N = 79)	
Total			
Median	0	0	0.48
Range	0-24.8	0-28.1	
Polymorphonuclear cells			
Median	0	0	0.90
Range	0-24.8	0-23.4	
Other leukocytes			
Median	0	0	0.31
Range	0-4.3	0-9.6	

* P value determined using the Mann-Whitney U-test.

in men with prostatodynia, but none of these differences was significant (Table 1). These findings did not change substantially when we used a number of other definitions for inflammation in the expressed prostatic secretions (e.g., $\geq 5.0 \times 10^5$ leukocytes/ml, $\geq 7.5 \times 10^5$ leukocytes/ml, $\geq 2.0 \times 10^6$ leukocytes/ml, or $\geq 2.5 \times 10^6$ leukocytes/ml) and/or the seminal fluid (e.g., $\geq 5.0 \times 10^5$ leukocytes/ml, $\geq 2.0 \times 10^6$ leukocytes/ml, $\geq 3.0 \times 10^6$ leukocytes/ml, $\geq 5.0 \times 10^6$ leukocytes/ml, ≥ 1 leukocyte/100 sperm, or ≥ 10 leukocytes/100 sperm; data not shown).

Of the 21 men with nonbacterial prostatitis, 6 (29%) had seminal inflammation and 15 (71%) had seminal leukocyte concentrations $< 10^6/\text{ml}$ (Fig. 1). Of the 79 men with prostatodynia, 17 (22%) had seminal inflammation and 62 (78%) had seminal leukocyte concentrations $< 10^6/\text{ml}$ (not significant).

Classified according to seminal fluid findings, 23 of the 100 men had seminal inflammation and 77 did not. Of the 23 men with seminal fluid inflammation, 6 (26%) had nonbacterial prostatitis, with a mean (\pm SD) leukocyte

Seminal Fluid Leukocytes in Men with Nonbacterial Prostatitis (NBP) and Prostatodynia (PD)

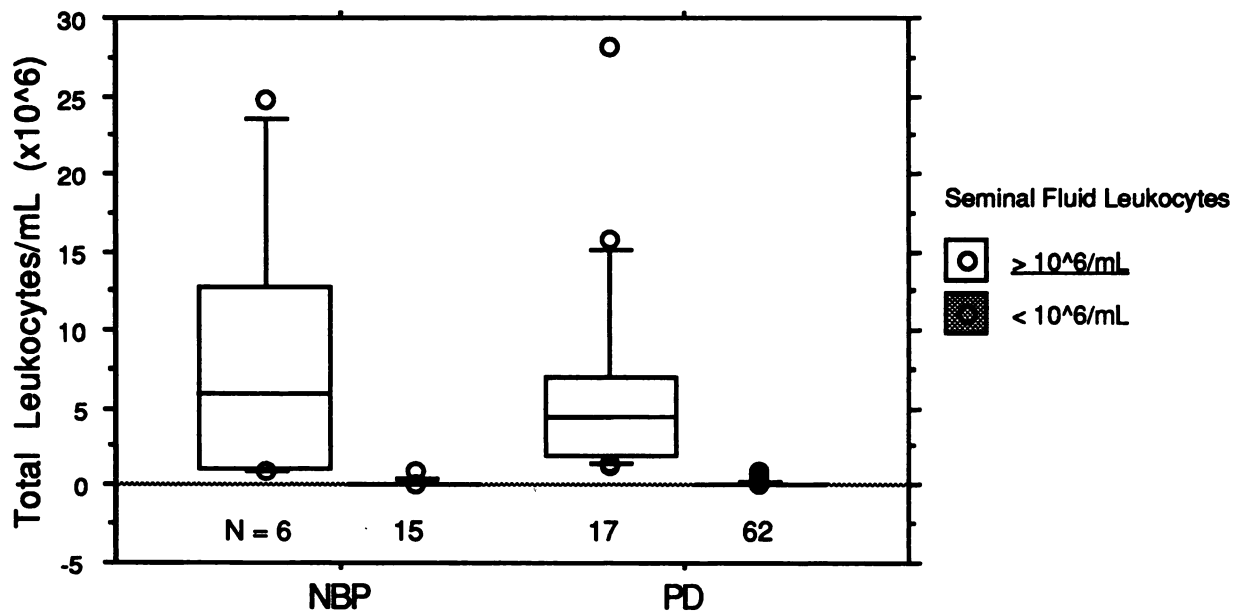


FIG. 1. Box diagram showing the median (line within box), 25th percentile (upper margin of box), 75th percentile (lower margin of box), SD (error bars), and outliers (circles). There was no statistically significant difference in the concentration of seminal leukocytes between the men with seminal inflammation who had NBP and those who had PD. There also was no significant difference in the concentration of leukocytes among men who did not have seminal inflammation between the men with NBP and those with PD.

Table 2. Seminal fluid parameters in 100 men with nonbacterial prostatitis and prostatodynia (classified by presence or absence of inflammation in expressed prostatic secretions)

Parameter	Nonbacterial prostatitis (N = 21)	Prostatodynia (N = 79)	Significance
Volume (ml)			
Median	2.4	2.5	0.88*
Range	0.5–7.8	0.3–6.5	
Sperm/ml ($\times 10^6$)			
Median	62.0	69.0	>0.99*
Range	0–425.0	0–888.0	
Total count ($\times 10^6$)			
Median	155.0	136.8	0.83*
Range	0–750	0–2,208.6	
Viscosity†			
Median	4	4	0.90‡
Range	2–4	1–4	
Motility (%)			
Median	45.0	60.0	0.08*
Range	0–94.0	0–100	
Subjective speed†			
Median	3	4	0.03‡
Range	0–4	0–4	
Forward progress†			
Median	3	3	0.23‡
Range	0–4	0–4	
Agglutination†			
Median	0	0	0.84‡
Range	0–1	0–4	
Normal morphology§	11/18 (61%)	31/68 (46%)	0.24‡

* Determined using the Mann-Whitney U-test.

† Scored on a scale of 0–4.

‡ Chi-square test.

§ Based on automated analysis using the Hamilton Thome IVOS computerized sperm analyzer and "Dimensions" program.

concentration of $8.6 \pm 9.4 \times 10^6/\text{ml}$ of semen, and 17 (74%) had prostatodynia, with a mean leukocyte concentration of $6.2 \pm 7.0 \times 10^6/\text{ml}$ of semen (not significant). Of the 77 men who did not have seminal inflammation, 15 (19%) had nonbacterial prostatitis, with a mean leukocyte concentration of $0.1 \pm 0.2 \times 10^6/\text{ml}$ of semen, and 62 (81%) had prostatodynia, with a mean leukocyte concentration of $0.1 \pm 0.2 \times 10^6/\text{ml}$ of semen (not significant).

Seminal Fluid Analysis in Men With Nonbacterial Prostatitis and Prostatodynia

Men with nonbacterial prostatitis had lower values for several parameters associated with motility (Table 2). The median proportion of motile sperm was 45% for the 21 men with nonbacterial prostatitis, compared to a median of 60% for the 79 men with prostatodynia ($P = 0.08$). The median sperm subjective speed score was 3 for the men with nonbacterial prostatitis, compared to a mean of 4 for the men with prostatodynia ($P = 0.03$). Mean forward progress was also lower for the men with nonbac-

terial prostatitis, although this difference did not approach statistical significance. There was no significant difference between men with nonbacterial prostatitis and prostatodynia in volume, sperm concentration, total sperm count, viscosity, agglutination, or the proportion of sperm with normal morphology.

Leukocyte Concentrations in Expressed Prostatic Fluid of Men With and Without Seminal Inflammation

We compared findings in expressed prostatic secretions and seminal fluid in our population of men with chronic nonbacterial prostatitis and prostatodynia. Among the 23 men with seminal inflammation, the median concentration of leukocytes was $4.4 \times 10^6/\text{ml}$ (range $1.0\text{--}28.1 \times 10^6/\text{ml}$), including a median of 2.1×10^6 polymorphonuclear cells (range $0\text{--}24.8 \times 10^6/\text{ml}$), and a median of 0 other leukocytes (range $0\text{--}9.6 \times 10^6/\text{ml}$). Among the 77 men without seminal inflammation, the median concentration of leukocytes was 0 (range $0\text{--}0.9 \times 10^6/\text{ml}$) in the seminal fluid, including a median of 0 polymorphonuclear cells (range $0\text{--}0.9 \times 10^6/\text{ml}$), and a median of 0 other leukocytes (range $0\text{--}0.8 \times 10^6/\text{ml}$). There was no significant difference in the concentrations of total, polymorphonuclear, or other leukocytes in the expressed prostatic secretions of men with and without seminal inflammation (Table 3).

Seminal Fluid Analysis in Men With and Without Seminal Inflammation

Men with chronic prostatitis and seminal inflammation had higher concentrations of sperm/ml (median $122 \times 10^6/\text{ml}$, compared to $55 \times 10^6/\text{ml}$ for men without seminal inflammation, $P < 0.0001$), higher total sperm counts (median 230×10^6 , compared to 55×10^6 for men without seminal inflammation, $P = 0.001$), and a higher proportion of normal morphology (62%, compared to 37% for men without seminal inflammation, $P < 0.05$; Table 4). There was no significant difference between men with and without seminal inflammation in volume, viscosity, motility, subjective speed, forward progress, or agglutination.

Discussion

Data obtained from this study argue against our hypothesis that men with nonbacterial prostatitis were more likely to have seminal inflammation than men with prostatodynia. In this carefully defined population, there was poor correlation between inflammation in the expressed prostatic secretions and inflammation in the semen. Although mean concentrations of seminal leukocytes were higher in men with nonbacterial prostatitis than in men

Table 3. Expressed prostatic fluid leukocyte concentrations in 100 men with nonbacterial prostatitis and prostatodynia

Expressed prostatic fluid leukocytes ($\times 10^6/\text{ml}$)	Population		Significance \ddagger
	Seminal inflammation* (N = 23)	No seminal inflammation (N = 77)	
Total			
Median	0.4	0.1	0.11
Range	0–24.0	0–36.0	
Polymorphonuclear cells			
Median	0.1	0.1	0.20
Range	0–10.0	0–92.1	
Other leukocytes			
Median	0	0	0.33
Range	0–24.0	0–29.0	

* Seminal inflammation was defined at $\geq 10^6$ leukocytes/ml of seminal fluid.

† P value determined using the Mann-Whitney U-test.

with prostatodynia, none of the differences approached significance. Overall, 29% of the 21 men with nonbacterial prostatitis had seminal inflammation, compared with 22% of the 79 men with prostatodynia.

Awareness of a number of issues helped us define the population of men in this study. Previous studies often included men with varied urological conditions or with

multiple causes of infertility. We evaluated men attending a chronic prostatitis clinic and excluded men with significant obstructive lesions or bacterial prostatitis, whether acute or chronic. These men had various genitourinary tract symptoms (to be described in detail in another publication) and were willing to undergo a series of uncomfortable evaluations. None of the 100 men in this study

Table 4. Seminal fluid parameters in 100 men with nonbacterial prostatitis and prostatodynia (classified by presence or absence of seminal inflammation)

Parameter	Seminal inflammation (N = 23)	No seminal inflammation (N = 77)	Significance
Volume (ml)			
Median	2.4	2.5	0.27*
Range	0.8–4.0	0.3–6.5	
Sperm/ml ($\times 10^6$)			
Median	122.0	55.0	<0.0001*
Range	15.0–614.0	0–888.0	
Total count ($\times 10^6$)			
Median	230.0	85.0	0.001*
Range	34.4–1,788.0	0–1,776.0	
Viscosity†			
Median	4	4	0.36‡
Range	1–4	1–4	
Motility (%)			
Median	60.0	50.0	0.73‡
Range	0–88.0	0–100	
Subjective speed†			
Median	4	3	0.80‡
Range	0–4	0–4	
Forward progress†			
Median	4	3	0.19‡
Range	0–4	0–4	
Agglutination†			
Median	0	0	0.26‡
Range	0–4	0–2	
Normal morphology§	13/21 (62%)	21/57 (37%)	0.048‡

* Mann-Whitney U-test.

† Scored on a scale of 0–4.

‡ Chi-square test.

§ Based on automated analysis using the Hamilton Thorne IVOS computerized sperm analyzer and "Dimensions" program.

were undergoing evaluation for infertility. In this regard our population corresponds to the most common clinical definition of prostatitis, rather than definitions based on seminal fluid evaluation.

The potential relationships between infection and most cases of prostatitis and seminal inflammation remain controversial. Some investigators had identified urethral pathogens, such as *C. trachomatis* and ureaplasmas, in $\geq 20\%$ of men with prostatitis syndromes (Bruce et al, 1981; Brunner et al, 1983; Weidner et al, 1985, 1988; Bruce and Reid, 1989). Isolation of *C. trachomatis* was reported from prostatic cells in 10 of 30 men with "non-acute abacterial prostatitis" and positive urethral cultures (Poletti et al, 1985). Persistent *C. trachomatis* infection (identified in urethral swabs after prostatic massage) was associated with treatment failure in 11 men with "urethroprostatitis" (Chiarini et al, 1994). *C. trachomatis* was associated with inflammation in pathological specimens removed for treatment of lower urinary tract obstruction (Shurbaji et al, 1988; Abdelatif et al, 1991). Ureaplasmas (Weidner et al, 1980, 1985) and *T. vaginalis* have also been associated with prostatitis syndromes (Kuberski, 1980). Other investigators (Shortliffe et al, 1992), including previous reports from our group (Berger et al, 1989; Krieger and Egan, 1991), seldom found *C. trachomatis* in men with symptoms of prostatitis and identified genital mycoplasmas as often in controls as in men with symptoms. Although antimicrobial agents are prescribed commonly for management of men with seminal inflammation and their sexual partners (El Bayoumi et al, 1982; Comhaire et al, 1986; Purvis and Christiansen, 1993; Branigan and Muller, 1994; Yanushpolsky et al, 1995), most studies did not include urethral cultures for organisms that may be associated with seminal inflammation. To limit the potential confounding role of urethral pathogens, we evaluated all subjects with cultures for *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis*, in addition to lower urinary tract localization studies for bacterial prostatitis. All subjects were also screened for urethritis by gram-stained urethral smears. Subjects with any of these organisms or evidence of urethritis were excluded from this study.

Lack of correlation between nonbacterial prostatitis and seminal inflammation might be explained by varying concentrations of leukocytes in expressed prostatic secretions or in semen over time. Two recent studies suggest that there is a high rate of resolution of seminal inflammation after 1 month, whether subjects were treated with antimicrobial agents or not (Branigan and Muller, 1994; Yanushpolsky et al, 1995). Wright et al (1994) conducted longitudinal studies of expressed prostatic secretion leukocytes in men with prostatitis. Resolution of inflammation in the expressed prostatic secretions occurred in only 9 (11%) of 79 men with "abacterial prostatitis" who had a mean follow-up of 7 visits over 40 months. In our study

most lower tract localization studies and seminal fluid analyses were done on the same day. Thus, spontaneous resolution of inflammation in either the expressed prostatic secretions or semen appears to be an unlikely explanation for our findings.

In this study, subjects with nonbacterial prostatitis had lower values for several parameters associated with motility, especially the median proportion of motile sperm (45% compared with 60% for men with prostatodynia, $P = 0.08$) and median sperm subjective speed (score of 3 compared with 4 for the men with prostatodynia, $P = 0.03$). Several earlier studies also evaluated seminal parameters in men with chronic prostatitis syndromes. Christiansen et al (1991) evaluated men with chronic abacterial prostatovesiculitis "verified" by transrectal ultrasonography (a method with low specificity) (Braeckman et al, 1991; Doble and Carter, 1989). Of 50 men with chronic prostatitis, 29 (58%) had seminal inflammation, compared with 4 (15%) of 25 controls. Weidner et al (1991) compared seminal findings in 102 men with nonbacterial prostatitis and 142 men with prostatodynia. They found no significant differences in the values of sperm density, motility, and morphology between the groups. Markedly different results were reported by Leib et al (1994), who evaluated 86 patients suffering from long-standing chronic abacterial prostatitis (not stratified by presence of inflammation in prostatic secretions). Sperm motility parameters, morphology characteristics, prostate markers, and leukocyte counts were all abnormal in the prostatitis group. Only 10% of the men with chronic prostatitis were classified as normospermic compared with 60% of controls (Leib et al, 1994). Schlegel (personal communication, 1992) also observed that treatment of chronic prostatitis often involves exposure to drugs that are toxic to sperm. To help control for this potential problem, men in this study were off all treatment for at least 4 weeks before their first visit and for 6–8 weeks before lower urinary tract localization and seminal fluid studies. The finding that nonbacterial prostatitis was associated with reduced sperm motility fits with a number of observations on the adverse effect of leukocytes on sperm structure or function (Berger et al, 1982; Maruyama et al, 1985; Anderson, 1990; Wolff et al, 1990; Aitken et al, 1991).

Does this study provide any insights into the functional significance of prostatitis syndromes in the male reproductive tract? Men with nonbacterial prostatitis and prostatodynia had similar concentrations of seminal leukocytes. However, the men with nonbacterial prostatitis had a lower proportion of motile sperm and lower sperm velocity (Table 2). Thus, nonbacterial prostatitis (defined by excessive leukocytes in the expressed prostatic secretions) may reduce sperm function independent of leukocyte counts in the semen. This observation was supported by seminal fluid findings in men with and without seminal

inflammation; the men with chronic prostatitis and seminal inflammation had significantly higher sperm counts and a higher proportion of normal sperm morphology than the men without seminal inflammation (Table 4). These findings suggest that investigating the expressed prostatic secretions may provide insights into reproductive function in men with no apparent abnormalities on routine seminal fluid analysis.

Does this study provide any insights into the source of leukocytes in the male reproductive tract? Potential sources of leukocytes include the urethra, paraurethral or Cowper's glands, prostate, seminal vesicles, vas deferens, epididymis, rete testis, and testis. None of the men in this study had evidence of urethral infection or inflammation, making a urethral source unlikely. Some men with no leukocytes in their expressed prostatic secretions had seminal inflammation, suggesting that such leukocytes could originate proximal to the prostate. Other men had inflammation in their expressed prostatic secretions without seminal inflammation. This finding might reflect differences in sampling of prostatic ducts with ejaculation and the massage procedure used to obtain expressed prostatic secretions. Our findings might also reflect differences in the functional state of leukocytes, such as activation status, or in the time that sperm are in contact with leukocytes from different sources in the male lower reproductive tract.

The methods used to enumerate leukocyte counts in semen are subject to some discussion (Purvis and Christiansen, 1993). Many studies used total "round cell" counts to evaluate seminal inflammation. However, distinguishing leukocytes from immature sperm is difficult, or impossible, without specialized staining procedures. For example, Politch et al (1993) found that peroxidase or immunohistology assays were effective but that total round cell counts proved of no value for enumerating seminal leukocytes. To avoid this issue we included Bryan-Leishman staining of all seminal fluid analyses to distinguish immature sperm from seminal leukocytes (Couture et al, 1976; Branigan and Muller, 1994).

Because sperm are the most common cellular elements, precise determination of seminal leukocyte counts may be subject to sampling errors. We evaluated the possibility of sampling errors by enumerating leukocytes per 500 sperm in seven cases of individuals who had nonbacterial prostatitis with inflamed expressed prostatic secretions but no seminal inflammation when leukocytes were counted per 100 sperm. All recounts were done in blinded fashion. Interestingly, none of these cases had >6 leukocytes/100 sperm when 500 sperm were counted, but all had leukocyte counts >0 (range 0.2–5.4/100 sperm). When calculated using the total sperm count, three of these seven cases had $>1.0 \times 10^6$ leukocytes/ml (1.21, 1.72, and 2.92×10^6 /ml). This finding suggests that stan-

dard methods for counting seminal leukocytes and defining significant inflammation lack sensitivity.

In contrast to the debate over methods used to count seminal leukocytes, there has been little discussion of the technique for counting leukocytes in expressed prostatic secretions. Direct microscopic examination of unstained preparations is the generally accepted method. Our experience in this study was that distinction of leukocytes from other elements in the semen is not straightforward in every case (data not shown). We are currently evaluating the utility of staining methods to increase the precision of this examination. Thus, lack of precision in counting leukocytes in prostatic secretions or semen may contribute to the apparent lack of correlation between inflammation at these sites.

In summary, we found poor correlation between standard definitions of inflammation in the expressed prostatic secretions and in the seminal fluid. Men with nonbacterial prostatitis had reduced quality of several seminal parameters associated with sperm motility. Our findings highlight clinical and technical issues that must be considered in designing future studies, especially enrollment of populations with defined clinical and microbiological characteristics. Studies should address inherent limitations in currently accepted methods for evaluating both the prostatic secretions and the seminal fluid. Rigorous methods are necessary for lower urinary tract localization and seminal fluid analyses, including special staining to distinguish immature sperm from seminal leukocytes. This study illustrates the importance of investigating multiple sites and samples, including the urethra, expressed prostatic secretions, and the seminal fluid, to achieve a comprehensive understanding of male reproductive function.

References

- Abdelatif OM, Chandler FW, McGuire BSJ. *Chlamydia trachomatis* in chronic abacterial prostatitis: demonstration by colorimetric *in situ* hybridization. *Hum Pathol* 1991;22:41–44.
- Aitken RJ, West K, Buckingham D. Leukocytic infiltration into the human ejaculate and its association with semen quality, oxidative stress, and sperm function. *J Androl* 1994;15:343–352.
- Anderson DJ. Cell-mediated immunity and inflammatory processes in male infertility. *Arch Immunol Ther Exp Warsz* 1990;38:79–86.
- Anderson RU, Weller C. Prostatic secretion leukocyte studies in nonbacterial prostatitis (prostatosis). *J Urol* 1979;121:292–294.
- Berger RE, Karp LE, Williamson RA, Koehler J, Moore DE, Holmes KK. The relationship of pyospermia and seminal fluid bacteriology to sperm function as reflected in the sperm penetration assay. *Fertil Steril* 1982;37:557–564.
- Berger RE, Krieger JN, Kessler D, Ireton RC, Close C, Holmes KK, Roberts PL. Case-control study of men with suspected chronic idiopathic prostatitis. *J Urol* 1989;141:328–331.
- Bowie WR, Wang SP, Alexander ER, Floyd J, Forsyth PS, Pollock HM, Lin JS, Buchanan TM, Holmes KK. Etiology of nongonococcal urethritis: evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *J Clin Invest* 1977;59:735–742.

- Braeckman JG, Figuera FC, Vanwaeyenbergh JG, Merckx LA, Keuppens FI. Reproducibility of transrectal ultrasound of prostatic disease. *Scand J Urol Nephrol (Suppl)* 1991;137:91-93.
- Branigan EF, Muller CH. Efficacy of treatment and recurrence rate of leukocytospermia in infertile men with prostatitis. *Fertil Steril* 1994;62:580-584.
- Bruce AW, Chadwick P, Willett WS, O'Shaughnessy M. The role of chlamydiae in genitourinary disease. *J Urol* 1981;126:625-629.
- Bruce AW, Reid G. Prostatitis associated with *Chlamydia trachomatis* in 6 patients. *J Urol* 1989;142:1006-1007.
- Brunner H, Weidner W, Schiefer HG. Studies on the role of *Ureaplasma urealyticum* and *Mycoplasma hominis* in prostatitis. *J Infect Dis* 1983;147:807-813.
- Chiarini F, Mansi A, Tomao P, Gentile V, De Marco F, Brunori S, Wongher L, Di Silverio F. *Chlamydia trachomatis* genitourinary infections: laboratory diagnosis and therapeutic aspects. Evaluation of *in vitro* and *in vivo* effectiveness of azithromycin. *J Chemother* 1994;6:238-242.
- Christiansen E, Tollefsrud A, Purvis K. Sperm quality in men with chronic abacterial prostatovesiculitis verified by rectal ultrasonography. *Urology* 1991;38:545-549.
- Comhaire FH, Rowe PJ, Farley TM. The effect of doxycycline in infertile couples with male accessory gland infection: a double blind prospective study. *Int J Androl* 1986;9:91-98.
- Couture M, Ulstein M, Leonard J, Paulsen CA. Improved staining method for differentiating immature sperm from white blood cells in human seminal fluid. *Andrologia* 1976;8:61-66.
- de la Rosette JJ, Hubregtse MR, Meuleman EJ, Stolk-Engelaar MV, Debruyne FM. Diagnosis and treatment of 409 patients with prostatitis syndromes. *Urology* 1993;41:301-307.
- Doble A, Carter SS. Ultrasonographic findings in prostatitis. *Urol Clin North Am* 1989;16:763-772.
- El Bayoumi MA, Hamada TA, El Mokaddem HH. Male infertility: etiologic factors in 385 consecutive cases. *Andrologia* 1982;14:333-339.
- Gonzales GF, Kortebani G, Mazzolli AB. Leukocytospermia and function of the seminal vesicles on seminal quality. *Fertil Steril* 1992;57:1058-1065.
- Kenny G. Mycoplasmas. In: Balows A, Hausler WJ Jr, Herrmann KL, Isenberg HD, Shadomy HJ, eds. *Manual of Clinical Microbiology*. 5th ed. Washington, DC: American Society for Microbiology; 1991: 478-482.
- Krieger JN, Egan KJ. Comprehensive evaluation and treatment of 75 men referred to chronic prostatitis clinic. *Urology* 1991;38:11-19.
- Krieger JN, Hooton TM, Brust PJ, Holmes KK, Stamm WE. Evaluation of chronic urethritis: defining the role for endoscopic procedures. *Arch Intern Med* 1988a;148:703-707.
- Krieger JN, McGonagle LA. Diagnostic considerations and interpretation of microbiological findings for evaluation of chronic prostatitis. *J Clin Microbiol* 1989;27:2240-2244.
- Krieger J, Tam M, Stevens C, Nielsen I, Hale J, Kiviat N, Holmes K. Diagnosis of trichomoniasis: comparison of conventional wet mount examination with cytologic studies, cultures and monoclonal antibody staining of direct specimens. *JAMA* 1988b;259:1223-1227.
- Kuberski T. *Trichomonas vaginalis* associated with nongonococcal urethritis and prostatitis. *Sex Transm Dis* 1980;7:135-136.
- Leib Z, Bartoov B, Eltes F, Servadio C. Reduced semen quality caused by chronic abacterial prostatitis: an enigma or reality? *Fertil Steril* 1994;61:1109-1116.
- Maruyama DKJ, Hale RW, Rogers BJ. Effects of white blood cells on the *in vitro* penetration of zona-free hamster eggs by human spermatozoa. *J Androl* 1985;6:127-135.
- Poletti F, Medici MC, Alinovi A, Menozzi MG, Sacchini P, Stagni G, Toni M, Benoldi D. Isolation of *Chlamydia trachomatis* from the prostatic cells in patients affected by nonacute abacterial prostatitis. *J Urol* 1985;134:691-693.
- Politch JA, Wolff H, Hill JA, Anderson DJ. Comparison of methods to enumerate white blood cells in semen. *Fertil Steril* 1993;60:372-375.
- Purvis K, Christiansen E. Infection in the male reproductive tract: impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 1993;16:1-13.
- Shortliffe LM, Sellers RG, Schachter J. The characterization of nonbacterial prostatitis: search for an etiology. *J Urol* 1992;148:1461-1466.
- Shurbaji MS, Gupta PK, Myers J. Immunohistochemical demonstration of chlamydial antigens in association with prostatitis. *Mod Pathol* 1988;1:348-351.
- Stamey TA. *Pathogenesis and Treatment of Urinary Tract Infections*. Baltimore: Williams and Wilkins, 1980.
- Stamm W, Tam M, Koester M, Cles L. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. *J Clin Microbiol* 1983;17:666-668.
- Weidner W, Brunner H, Krause W. Quantitative culture of *Ureaplasma urealyticum* in patients with chronic prostatitis or prostaticos. *J Urol* 1980;124:622-625.
- Weidner W, Jantos C, Schiefer HG, Haidl G, Friedrich HJ. Semen parameters in men with and without proven chronic prostatitis. *Arch Androl* 1991;26:173-183.
- Weidner W, Krause W, Schiefer HG, Brunner H, Friedrich HJ. Ureaplasma infections of the male urogenital tract, in particular prostatitis, and semen quality. *Urol Int* 1985;40:5-9.
- Weidner W, Schiefer HG, Krauss H. Role of *Chlamydia trachomatis* and mycoplasmas in chronic prostatitis: a review. *Urol Int* 1988;43:167-173.
- Wolff H, Politch JA, Martinez A, Haimovici F, Hill JA, Anderson DJ. Leukocytospermia is associated with poor semen quality. *Fertil Steril* 1990;53:528-536.
- World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. 3rd ed. Cambridge, UK: Cambridge University Press; 1992.
- Wright ET, Chmiel JS, Grayhack JT, Schaeffer AJ. Prostatic fluid inflammation in prostatitis. *J Urol* 1994;152:2300-2303.
- Yanushpolsky EH, Politch JA, Hill JA, Anderson DJ. Antibiotic therapy and leukocytospermia: a prospective, randomized, controlled study. *Fertil Steril* 1995;63:142-147.