# Pilot and Feasibility Study of Serum Chemokines as Markers to Distinguish Prostatic Disease in Men With Low Total Serum PSA

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**BACKGROUND.** The incidence and prevalence of both benign prostatic hypertrophy (BPH) and prostate cancer (PCa) increase with the aging process. Our laboratory recently showed that the chemokines CXCL5 and CXCL12, which normally function as inflammatory mediators, are secreted at higher levels by aging prostate stromal fibroblasts and elicit proliferative responses from both prostate stromal fibroblast and epithelial cells. Because both CXCL5 and CXCL12 are secreted molecules, we hypothesized that their levels in patient serum might serve as biomarkers to distinguish between BPH and PCa.

**METHODS.** Serum CXCL5 and CXCL12 levels were determined using sandwich ELISAs for 51 men demonstrating low serum PSA values of $\leq$10 ng/ml who underwent diagnostic needle biopsy for the detection of PCa. The bivariate relationship of circulating chemokine levels, age, and disease status in the prostate was tested using the Wilcoxon rank-sum test.

**RESULTS.** Total serum CXCL12 levels were significantly higher for men who were biopsy positive compared to those who were biopsy negative for cancer and histological prostatitis ($P = 0.050$). Among men who were biopsy negative for PCa, total serum CXCL5 levels were inversely associated with prostate volume and were significantly higher in men with concomitant BPH and histological prostatitis compared to those without evidence of prostatic disease ($P < 0.003$).

**CONCLUSIONS.** The results of this pilot and feasibility study suggest that serum or plasma CXCL5 and CXCL12 levels may potentially distinguish between BPH and PCa among patients presenting with low serum PSA, and may be useful toward facilitating decisions to perform diagnostic needle biopsy in this patient population. *Prostate 68: 442–452, 2008.*



*KEY WORDS:* **CXCL12; CXCL5; serum; biomarker; prostate**

## INTRODUCTION

Benign Prostatic Hypertrophy (BPH, also referred to as benign prostatic enlargement) is one of the most common benign proliferative conditions associated with aging in men [1–3]. BPH is pathologically characterized by cellular proliferation of the epithelial and stromal elements primarily within the periurethral, or transitional zone, region of the prostate gland [1–3,4]. In a survey of 1,709 men without cancer reported by the Massachusetts Male Aging Study, the

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frequency of clinical BPH (defined in terms of frequency/difficulty with urinating and evidence of an enlarged/swollen prostate) rose from 8.4% in men 38–49 years of age to 33.5% in men aged 60–70 years ($P < 0.001$) [2]. The incidence of BPH in association with aging is much higher than the incidence of symptom-inducing clinical BPH, for example, lower urinary tract symptoms (LUTS) [5]. However, LUTS may progress to bladder outlet obstruction-related disorders, including acute urinary retention (AUR) that may require therapeutic and/or surgical intervention [6]. Aging is also a risk factor in the development of prostatic malignancies as the American Cancer Society estimates that the probability of developing an invasive prostate cancer (PCa) in 2007 is 0.01% between birth and the fourth decade of life, but rises to 2.59% in the fifth decade, 7.03% in the sixth decade, 13.83% in the seventh decade, and 17.12% in the eighth decade of life for American men [7]. Clearly, age is a major risk factor for the development of both BPH and PCa.

The biological mechanisms responsible for the observed increase in age-associated risk for the development of BPH and PCa are poorly understood. The finding that chemokine-type inflammatory mediators are secreted consequent to aging and promote proliferative responses from both non-transformed and transformed prostate epithelial cells suggests that inflammation and inflammatory responses might play a causal role in the development of both BPH and PCa [8]. Indeed, the frequent observation of inflammatory infiltrate in the prostate coincident with BPH or PCa has provoked intense interest in the potential role of inflammatory mediators in the etiology of both diseases. For example, Nickel et al. described studies that examined sectioned transurethral resection of the prostate (TURP) specimens from 80 consecutive patients with a diagnosis of BPH but no history or symptoms of prostatitis. Inflammatory cells were detected in 90% of specimens examined, regardless of whether the patient had been catheterized for urinary retention prior to TURP or whether bacterial growth resulted from cultured specimens. They concluded that prostatic inflammation is an extremely common histological finding in patients with BPH who have no symptoms of prostatitis, though the clinical significance of asymptomatic chronic prostatitis associated with BPH had yet to be determined [9]. In another study, Gerstenbluth et al. identified pervasive chronic prostatitis in whole mount radical prostatectomy specimens from a series of 40 consecutive patients with clinically localized PCa. Although inflammation was associated with both BPH and cancer, it was observed more frequently with BPH. They concluded that these findings indirectly supported a potential role for inflammation in the pathogenesis of BPH [10]. Data recently reported by Roehrborn obtained from examining baseline prostate biopsies in a subgroup of 1197 randomly selected patients in the Medical Therapy of Prostatic Symptoms (MTOPS) study demonstrated chronic inflammatory infiltrate in 30–60% of men with BPH. Patients with chronic inflammatory infiltrate had larger prostate volumes and demonstrated significantly more clinical progression and AUR than those who had no inflammation [11,12].

With regard to the role of inflammation in prostate tumorigenesis, De Marzo et al. have identified a type of hyperproliferative lesion in the prostate that is associated with inflammation and is morphologically similar to prostatic atrophy termed proliferative inflammatory atrophy, or PIA [13]. This group also showed that high-grade prostatic intraepithelial neoplasia (PIN) is often observed in proximity to PIA, and that morphologic transitions between high-grade PIN and PIA occur frequently within the same acinus/duct. These and other studies suggest a model in which proliferative epithelium associated with inflammation may progress to PIN and/or adenocarcinoma [13–15]. Epidemiological studies also have noted an increased risk for PCa among men with a history of prostatic inflammation [16]. Though only associative, these studies are suggestive of a link between inflammatory processes and prostate tumorigenesis.

Detection rates for PCa in men demonstrating total serum PSA (tPSA) values greater than 10 ng/ml are typically 70% or higher when combined with findings of abnormal digital rectal exam (DRE) or with histological evidence based on >6 needle biopsy specimens [17,18]. These rates, however, are much lower for men demonstrating tPSA values of <10 ng/ml. For example, malignant glands were detected on needle biopsy for ~30% of men whose tPSA values were between 4 and 10 ng/ml, and tumor detection fell to 21–23% among men with detectable tPSA values of <4 ng/ml [19–21]. This suggests that factors other than cancer may contribute to the elevation in PSA in the serum. Indeed, elevated serum tPSA values correlate directly with histological evidence of inflammation on needle biopsy in patients asymptomatic for prostatitis [22]. Another study evaluating patients enrolled in the Chronic Prostatitis Cohort Study and age-matched controls found that total and other forms of serum PSA was elevated in men diagnosed with chronic prostatitis/chronic pelvic pain syndrome. Importantly, this study determined that total and other forms of serum PSA alone did not demonstrate sufficient sensitivity and specificity for use as diagnostic markers for chronic prostatitis/chronic pelvic pain syndrome [23]. Lastly, larger prostate volume may contribute to elevated tPSA values in the absence of cancer. A recent study showed that a smaller prostate volume is the strongest predictor

of cancer detection in men exhibiting tPSA levels in the 2.0–9.0 ng/ml range, suggesting that tPSA is less useful for the prediction of cancer in men with concurrent BPH [24]. Several studies have shown that serum tPSA values increase concomitantly with patient age in parallel with increased incidence of BPH [25–29].

Taken together, these studies show that additional serum biomarkers would be very valuable to distinguish between prostatic diseases in men exhibiting serum PSA values of <10 ng/ml. Studies recently reported from our laboratory showed that aging prostate stromal cells cultured in vitro secrete CXC-type chemokines, which act as potent growth factors that promote the proliferation of both non-transformed and transformed prostatic epithelial cells. These studies potentially link stromally secreted chemokine-type inflammatory mediators consequent to aging with benign and malignant proliferative diseases of the prostate [8,30]. Based on these findings, we conducted a pilot and feasibility study to test the hypothesis that the serum concentrations of specific CXC-type chemokines may provide objective criteria to facilitate a differential diagnosis of BPH or PCa in men with low (≤10 ng/ml) serum PSA.

## METHODS

### Patient Population and Demographics

The patient population was drawn from men referred to the University of Michigan Health System (UMHS) with an indication for prostate biopsy, for example, rising or elevated total PSA, abnormal DRE, high-grade PIN (HGPIN), or atypical small acinar proliferation (ASAP) on prior biopsy, positive family history of PCa, and known PCa on watchful waiting. As not all of these men went on to have surgical treatment, only those with radical prostatectomy at our center had additional pathological annotation beyond the biopsy. Patients presenting for a prostate biopsy were approached to participate in an on-going prospective Prostate Biopsy Clinical database/Tissue Bank study that enables several studies with Institutional Review Board approval, including the prostate biopsy referral database, the Early Detection Research Network (EDRN), and the study reported here. Over the 12-month period between August 2005 and September 2006, 133 patients consented to the collection and use of clinical data and tissue (serum, urine, prostate tissue). The final study population of 51 patients was selected for those exhibiting pre-biopsy tPSA values of ≤10 ng/ml (determined using the Abbott AxSYM polyclonal-monoclonal immunoassay (Abbott Diagnostics, Abbott Park, IL)), that approximated the observed frequencies of biopsy-verified PCa (36%) and histological prostatitis (26%) in the larger EDRN study population, and that permitted examination of equivalent cases of high-volume disease within both the biopsy-negative and -positive cases.

### Collection of Clinical Data

Clinical and demographic data were collected from the electronic medical record (UMHS Careweb) or hard copy medical records for all subjects. This data included patient age, date of biopsy, physician, serum PSA levels, medical history, comorbid conditions, medications, physical examination including DRE findings, prior history of prostate biopsies, cost related to the procedure, complications, (AUA Symptom Score/satisfaction with voiding situation), prostate size based on the transrectal ultrasound (TRUS), and findings from the prostate biopsies. Also, as patients were seen in follow-up, any changes in disease status or additional diagnostic testing were added to the database.

Prostate volume data were gathered during a standard TRUS examination performed using a 7.5 MHz biplanar endorectal probe. In addition to assessing the echogenic pattern of the prostate gland, three measurements were made to calculate total prostatic volume. The anterior–posterior (AP) and transverse (TR) diameters were measured at their respective maximal dimensions, whereas the superior–inferior (SI) diameter was measured as the maximal length from the base to the apex of the prostate in the midline sagittal plane. Total prostate volume was estimated by static images using the formula for a prolate ellipsoid, volume $= \pi/6(TR \times AP \times SI)$.

LUTS severity information was gathered using the American Urological Association Symptom Index (AUASI) indices. These are generated from data acquired from a patient-administered questionnaire, the eight item validated American Urological Association Symptom Score, which assesses the severity of lower urinary symptoms in men that are most often attributed to prostate disease. This survey is self-completed by the patient prior to the prostate biopsy. AUASI scores of 1–7 indicate none/mild LUTS, 8–19 indicate moderate LUTS, and 20–35 indicate severe LUTS [31].

### Collection of Clinical Specimens

Serum samples were collected just prior to prostate needle biopsy in order to obviate any potential surgical- or trauma-induced impact on circulating chemokine or other protein levels in this patient group. As standard procedure, all patients were advised to refrain from taking oral non-steroidal anti-inflammatory drugs (NSAIDs) and other over-the-counter medications for

1 week prior to biopsy. This served to minimize or obviate potential medication-mediated fluctuations in serum chemokine levels. For all patients, blood was drawn into two 30cc heparinized tubes and one 15cc EDTA tube, which were placed on ice and processed within 12 hr. The blood in the heparinized tubes was transferred into 15 ml tubes, centrifuged at 2,500 rpm for 10 min, and stored in 200 ul aliquots in 0.5 ml cryovials (Sarstedt) at −80°C. The blood from the EDTA tube was diluted with an equal volume of PBS and subjected to Ficoll Hypaque density gradient centrifugation to separate the lymphocyte granulocyte layer (''buffy coat'') and plasma. The plasma layer was carefully removed to a 15 ml tube and centrifuged at 4,000 rpm for 10 min at 4°C to remove platelets and all cellular contaminants. The platelet-free plasma was stored at −80°C in 200 ul aliquots in 0.5 ml cryovials (Sarstedt).

Prostate biopsies were typically performed transrectally using a 12-core extended biopsy template with traditional paramedian sextant biopsies plus additional needle cores directed more laterally [32]. All needle biopsies containing malignant glands were quantitated as to percent of malignant tissue, and further evaluation of perineural invasion or extraprostatic extension was provided. All needle biopsies are evaluated for the presence of HGPIN/ASAP, inflammation (acute and chronic), hyperplasia, or other histopathologies. When PCa was identified, each set of needle biopsies was given an overall Gleason grade based on the evaluation of the entire tumor.

### Definitions of Prostatic Disease Status and Study Groups

These data are summarized in Table I. Disease status was carefully defined in the study group, as delineated below. It should be noted that some patients included in the study were characterized by more than one disease status.

**No Disease.** Criteria: No finding of cancer on prostate biopsy. Negative diagnosis for histological prostatitis based on negative findings for acute and/or chronic inflammatory infiltrate on prostate biopsy or history of clinically diagnosed prostatitis. Prostate volume $\leq$ 37.5 g on TRUS. Prostate biopsy specimens evaluated as normal benign. PSA values $\leq 10$ ng/ml. This comprised 13/51 (25%) of the total patient population examined in this study.

**Histological Prostatitis.** Criteria: Histological diagnosis of acute and/or chronic inflammatory infiltrate for one or more prostate biopsy specimens. This comprised 16/51 (31%) of the total patient population examined in this study. Fifteen of these patients were biopsy negative for cancer, and one was biopsy positive. NB: none of the 51 patients in the patient population examined in these studies described a clinical diagnosis for prostatitis.

**BPH.** Criteria: Evidence for enlarged prostate. The median prostate volume for the 51 patients included in this study was 37.5 g. Therefore, this value was used to define prostates as low volume ($\leq$37.5 g) or high volume (>37.5 g), roughly equivalent to measures described as high volume and consistent with BPH in the literature [24]. Men with BPH comprised 25/51 (49%) of the total patient population examined in this study, 17/37 (46%) of biopsy-negative patients, and 8/14 (57%) of biopsy-positive patients. Although the AUASI was recorded for all patients, it was not used to define BPH.

**Cancer.** Criteria: Histological diagnosis of malignant glands from prostate biopsy. This comprised 14/51 (27%) of the patient population examined in this study, which approximated the proportion of patients (1/3, 33%) with biopsy-verified cancer within the larger patient population comprising the EDRN study. Five board-certified pathologists were involved in the histologic diagnoses of the prostate biopsies. The number of positive biopsies among these 14 patients varied from 1 to 7, with a mean (and median) of two positive biopsies per patient, with a mean (and median) Gleason score of 6. The subsequent treatment courses for these patients varied widely, with five patients undergoing laparoscopic or radical retropubic prostatectomy, three patients undergoing external beam radiation or brachytheraphy, four patients under watchful waiting, and two patients who did not return for treatment to the University of Michigan.

### ELISA Assays

Circulating serum CXCL5 (ENA-78) or CXCL12 (SDF-1) chemokine levels were assessed using 50 ul frozen serum or plasma per direct sandwich ELISA in duplicate using the Human CXCL5/ENA-78 DuoSet kit DY254 or the Human CXCL12/SDF-1 alpha capture antibody MAB350, detection antibody BAF310, and standard 350-NS ELISA reagents (R&D Systems). Measures within each patient group were regarded as biological replicates and permitted statistical comparisons between groups. For all ELISAs, a standard curve was generated with the provided standards and utilized to calculate the quantity of chemokine in the sample tested. These assays provide measures of chemokines concentration with excellent reproducibility, with replicate measures characterized by standard

**TABLE I. Summary of Patient Demographic, Clinical, and Histological Data**

| | | Volume (g) | | | Age (years) | | | PSA (ng/ml) | | | AUASI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient category | N | Median | Mean (SD) | *P*-value | Median | Mean (SD) | *P*-value | Median | Mean (SD) | *P*-value | Median | Mean (SD) | *P*-value |
| All patients | 51 | 37.5 | 45.4 (20.9) | | 59.0 | 59.5 (9.1) | | 4.2 | 4.5 (2.2) | | 9.0 | 9.4 (5.6) | |
| By subcategory | | | | | | | | | | | | | |
| Low volume (≤37.5 g) | 26 | 30.0 | 29.6 (5.6) | 0.000 | 59.5 | 60.0 (10.1) | 0.792 | 3.2 | 3.6 (2.3) | 0.000 | 6.5 | 8.2 (6.4) | 0.033 |
| High volume (>37.5 g) | 25 | 55.1 | 61.8 (18.1) | | 59.0 | 59.0 (8.1) | | 5.4 | 5.4 (1.6) | | 11.0 | 10.6 (4.6) | |
| Without prostatitis | 35 | 34.4 | 39.4 (16.5) | 0.008 | 61.0 | 61.9 (8.9) | 0.003 | 4.2 | 4.4 (2.1) | 0.662 | 9.0 | 9.8 (5.7) | 0.483 |
| With prostatitis | 16 | 53.1 | 58.5 (24.0) | | 54.0 | 54.1 (7.2) | | 4.1 | 4.8 (2.3) | | 8.0 | 8.4 (5.4) | |
| Low volume, without prostatitis | 22 | 30.0 | 29.6 (5.6) | 0.915 | 61.0 | 61.6 (9.9) | 0.039 | 3.3 | 3.7 (2.3) | 0.499 | 7.5 | 9.3 (6.4) | 0.024 |
| Low volume, with prostatitis | 4 | 29.9 | 29.1 (6.5) | | 51.0 | 51.0 (6.2) | | 2.4 | 3.2 (1.9) | | 2.0 | 3.0 (2.8) | |
| High volume, without prostatitis | 13 | 53.7 | 55.8 (15.8) | 0.135 | 62.0 | 62.5 (7.3) | 0.021 | 5.7 | 5.5 (0.9) | 0.241 | 11.0 | 10.8 (4.6) | 0.816 |
| High volume with prostatitis | 12 | 78.7 | 68.3 (18.6) | | 55.5 | 55.2 (7.5) | | 4.5 | 5.3 (2.2) | | 9.0 | 10.4 (4.8) | |
| Patients without cancer | 37 | 37.0 | 46.4 (21.7) | | 57.0 | 58.0 (9.5) | | 3.9 | 4.4 (2.2) | | 9.0 | 9.8 (5.9) | |
| By subcategory | | | | | | | | | | | | | |
| Low volume | 20 | 31.3 | 29.9 (6.0) | 0.000 | 57.0 | 58.6 (10.6) | 0.915 | 3.0 | 3.4 (1.9) | 0.001 | 8.5 | 9.6 (6.8) | 0.557 |
| High volume | 17 | 60.0 | 65.8 (16.5) | | 57.0 | 57.4 (8.3) | | 5.1 | 5.5 (1.9) | | 9.0 | 10.0 (5.0) | |
| Patients with cancer | 14 | 40.0 | 42.7 (19.4) | | 61.5 | 63.4 (6.7) | | 5.0 | 4.9 (2.2) | | 6.5 | 8.5 (4.9) | |
| By subcategory | | | | | | | | | | | | | |
| Low volume | 6 | 27.8 | 28.6 (4.8) | 0.002 | 62.0 | 64.8 (6.5) | 0.603 | 3.8 | 4.3 (3.4) | 0.174 | 4.0 | 4.2 (1.2) | 0.003 |
| High volume | 8 | 48.5 | 53.2 (19.6) | | 61.5 | 62.3 (7.2) | | 5.7 | 5.2 (0.8) | | 11.5 | 11.8 (3.8) | |

deviations from the mean on the order of 1–2%. Measures for each chemokine were performed for all samples simultaneously to avoid potential ''batch effect'' variations in measurement.

### Statistical Analysis

The bivariate relationship of circulating chemokine levels, age, and disease severity (gland volume, baseline PSA, and AUASI) with disease status was tested in this small patient population using the Wilcoxon rank-sum test. Separate tests were performed for each definition of disease status (e.g., cancer vs. no cancer, low volume vs. high volume, etc) in pairwise fashion. Sample size prevented the ability to adjust for other factors in a multivariable analysis or to construct reliable receiver operator curve (ROC) analyses. All tests were performed using SAS v9.1.3 at the 5% significance level.

## RESULTS

### Clinico-Pathologic Characteristics of the Patient Population

The study population of 51 patients was selected for those cases exhibiting pre-biopsy tPSA values of $\leq$10 ng/ml, that roughly mirrored the observed frequencies of biopsy-verified PCa (36%) and histological prostatitis (26%) in the larger EDRN study population, and would permit examination of equivalent cases of high-volume disease (BPH) within both the biopsy-negative and -positive cases. Although all of the patients included in this study exhibited pre-biopsy tPSA values of $\leq$10 ng/ml, these values were significantly higher for men with BPH compared to those with smaller prostates ($P < 0.001$). Prostate volume itself varied concordantly with biopsy-diagnosed histological prostatitis (acute, chronic, or both) such that prostates without evidence of histological prostatitis were significantly smaller, with a median volume of 34.4 g, than prostates with evidence of histological prostatitis, with a median volume of 53.1 g ($P = 0.008$). Men with evidence of histological prostatitis on needle biopsy were significantly younger than those without evidence of histological prostatitis, with a median age of 54.0 compared to 61.0 years, respectively ($P = 0.003$). This relationship held both in the presence ($P = 0.021$) or absence ($P = 0.039$) of concurrent prostatic enlargement. Men that were biopsy positive for cancer were older (median age 61.5 years) than men that were biopsy-negative cancer for cancer (median age 57.0 years) but this difference was not significant ($P = 0.061$). Results from completion of the AUASI questionnaire for men without cancer demonstrated similar scores consistent with moderate symptoms for patients with smaller volume prostates (median score 8.5) or with prostatic enlargement (median score 9.0) ($P = 0.557$). However, men with cancer reported significantly higher scores consistent with moderate symptoms (median score 11.5) concordant with prostatic enlargement compared to men with smaller prostates who reported scores consistent with none or mild symptoms (median score 4.0) ($P = 0.003$). The presence or absence of biopsy-diagnosed histological prostatitis was not associated with significant differences in AUASI scores among the patient population as a whole or among men with or without cancer and/or prostatic enlargement in particular. These data are summarized in Table I.

### Serum CXCL5 and CXCl2 Levels are Concordant With the Presence of Prostatic Disease

Because CXCL5 and CXCL12 are secreted molecules and were found to be associated with aging and prostate cellular proliferation, we hypothesized that their levels in patient serum might, singly or in combination, serve as biomarkers to distinguish between BPH and PCa. To test this, ELISAs for CXCL5 and CXCL12 were performed on whole serum from 51 men enrolled in the EDRN study as described above. The ELISA analyses showed that total serum CXCL12 levels were significantly higher for patients exhibiting biopsy-verified cancer compared to men with who were biopsy negative for cancer and histological prostatitis ($P = 0.050$) (Table II and Fig. 1A). When evaluated according to prostate volume, serum CXCL12 levels were found to be lowest among men without BPH, cancer, or histological evidence of prostatitis. Moreover, serum CXCL12 levels were significantly different between men with no evidence of prostatic disease and men with biopsy-verified cancer ($P = 0.047$) (Table II and Fig. 1C). Thus, serum CXCL12 clearly distinguished men without prostatic disease from those with cancer, and this predictive value was most significant among men without BPH.

When analyzed without regard to prostate volume, total serum CXCL5 levels were unable to definitively distinguish between men with or without biopsy-diagnosed cancer (Table II and Fig. 1B). However, total serum CXCL5 levels were clearly inversely associated with prostate volume, with median values almost threefold higher in men with low volume prostates compared to men with BPH but no other evidence of prostatic disease (Table II and Fig. 1C). Among men with BPH who were biopsy negative for cancer, total serum CXCL5 levels were significantly higher in men with concomitant histological prostatitis compared to those without other evidence of disease ($P < 0.003$) (Table II and Fig. 1C). Thus, serum CXCL5 levels were

**TABLE II. Summary of Serum CXCL5 and CXCL12 Levels (ng/ml) in the Study Population**

| Comparison | N | CXCL5 | | | | CXCL12 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Median | *P*-value | Mean | SD | Median | *P*-value |
| With cancer: low and high volume compared to | 14 | 0.88 | 0.30 | 0.91 | | 1.17 | 1.03 | 0.82 | |
| Without cancer: all | 37 | 1.01 | 0.72 | 0.80 | 0.78 | 0.74 | 0.54 | 0.54 | 0.15 |
| Without cancer: all prostatitis | 15 | 0.68 | 0.25 | 0.77 | *0.06* | 1.05 | 0.61 | 1.27 | 0.98 |
| Without cancer: acute prostatitis only | 11 | 0.69 | 0.24 | 0.77 | 0.10 | 1.10 | 0.62 | 1.27 | 0.98 |
| Without cancer: no prostatitis | 26 | 1.14 | 0.81 | 1.01 | 0.64 | 0.58 | 0.43 | 0.49 | **0.05** |
| Without cancer: low volume only, no prostatitis | 13 | 1.39 | 0.86 | 1.24 | 0.12 | 0.55 | 0.38 | 0.39 | **0.05** |
| With cancer, high volume compared to | 8 | 1.05 | 0.18 | 1.03 | | 1.06 | 1.10 | 0.56 | |
| Without cancer: high volume, all prostatitis | 8 | 0.65 | 0.19 | 0.72 | **0.00** | 1.19 | 0.68 | 1.32 | 0.71 |
| Without cancer, high volume, no prostatitis | 9 | 0.67 | 0.44 | 0.40 | *0.06* | 0.65 | 0.53 | 0.54 | 0.74 |
| With cancer, low volume compared to | 6 | 0.66 | 0.29 | 0.67 | | 1.33 | 1.00 | 1.19 | |
| Without cancer: low volume, all prostatitis | 3 | 0.81 | 0.36 | 1.00 | 0.70 | 0.87 | 0.46 | 0.91 | 0.52 |
| Without cancer: low volume, no prostatitis | 17 | 1.39 | 0.86 | 1.24 | *0.06* | 0.55 | 0.38 | 0.39 | **0.05** |

Bold values indicates *P*-value associated with statistical significance ($P < 0.05$).

progressively elevated in, and predictive for, histological prostatitis and PCa concurrent with BPH.

### Comparison of Chemokine Measurements in Serum or Plasma

Because CXCR4 (the receptor for CXCL12) is expressed on platelets, and CXCL5 can be released from activated platelets, the CXCL12 and CXCL5 levels were measured by ELISA (as described above) and compared in both serum and platelet-free plasma from 6 of the 51 patients examined in this study. These assays showed that ELISA-derived values for CXCL12 were almost an order of magnitude lower, but parallel, in serum compared to plasma samples from the same patients (Fig. 1D). With the exception of values for one patient (patient ''B'' in Fig. 1D), ELISA-derived values for CXCL5 were in good agreement between serum and plasma samples from the same patients. For both CXCL5 and CXCL12, however, the majority of ELISA-derived values were higher in plasma compared to serum samples, suggesting that plasma may provide a more sensitive means for the quantitation of these chemokines than serum.

## DISCUSSION

The intent of the pilot and feasibility study reported here was to determine whether the serum levels of particular chemokine-type proteins secreted by aging prostate stroma may be predictive for either benign or malignant prostatic disease. The results of these studies showed that serum CXCL12 levels were significantly higher for patients exhibiting biopsy-verified cancer compared to men with who were biopsy negative for cancer and histological prostatitis. They also showed that serum CXCL5 levels were progressively elevated in men with histological prostatitis and PCa concurrent with BPH. Thus, serum levels of CXCL5 and CXCL12 differentially distinguished between BPH, histological prostatitis, and PCa in the small patient population examined in these studies.

These results are largely consistent with other studies in the literature suggesting that the secretion of particular cytokines and chemokines may be associated with benign and malignant proliferative disease. For example, Veltri et al. showed that serum levels of the chemokine IL-8 (CXCL8) provided an adjunct to serum PSA to distinguish between prostatic diseases [33]. Androgen-responsive LNCaP and LAPC-4 cells forced to over-express IL-8 exhibited reduced dependence on androgen for growth, decreased sensitivity to the anti-androgen, bicalutamide, and increased motility and invasiveness in vitro,

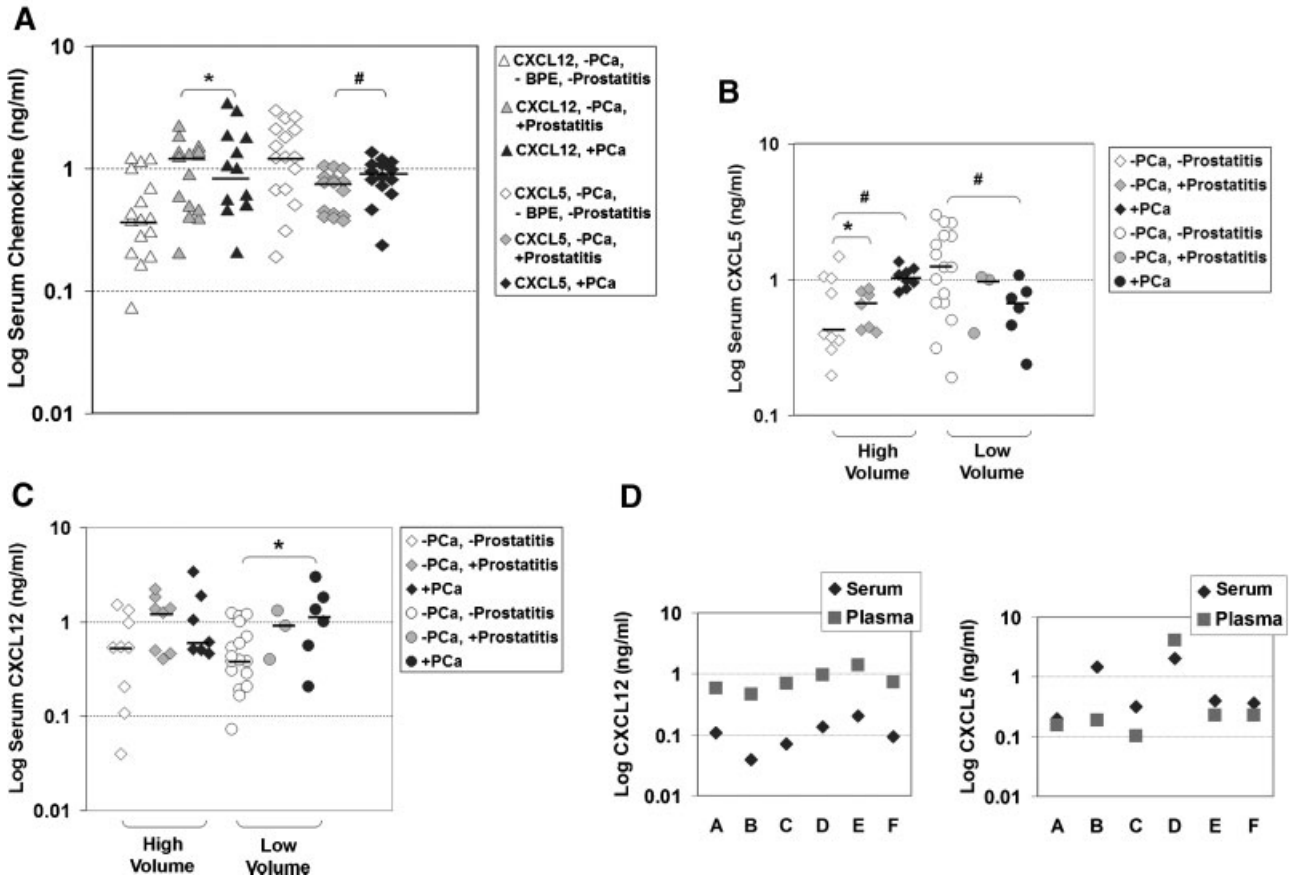


**Fig. 1.** **A**: Log serum CXCL5 or CXCLI2 (ng/ml) differs depending on prostate disease status. ELISA-derived values for serum CXCLI2 for men without evidence of prostatic disease (white triangles), men without cancer but with histological prostatitis (gray triangles) and men with prostate cancer (black triangles), as well as the log serum CXCL5 for men without evidence of prostatic disease (white diamonds), men without cancer but with histological prostatitis (gray diamonds), and men with prostate cancer (black diamonds) are shown on a logarithmic scale. Significant differences ($P < 0.050$) are indicated by *, trends ($0.065 < P < 0.050$) by #. **B**: Log serum CXCL5 (ng/ml) relevant to prostate volume. ELISA-derived values for serum CXCL5 for men with low volume ($\leq$37.5 g) (circles) or high-volume ($>$37.5 g) (diamonds) prostates without cancer or histological prostatitis (white), without cancer but with histological prostatitis (gray), or with cancer (black) are shown on a logarithmic scale Differences between groups that achieved statistical significance ($P < 0.05$) are indicated by *; trends by #. The data represented here are drawn from Table II. **C**: Log serum CXCLI2 (ng/ml) relevant to prostate volume. ELISA-derived values for serum CXCLI2 for men with low volume ($\leq$37.5 g) (circles) or high-volume ($>$37.5 g) (diamonds) prostates without cancer or histological prostatitis (white), without cancer but with histological prostatitis (gray), or with cancer (black) are shown on a logarithmic scale. Differences between groups that achieved statistical significance ($P < 0.05$) is indicated by *; trends by #. The data represented here is drawn from Table II. **D**: Log chemokine values in serum and plasma. ELISA-derived values for CXCLI2 (left) or CXCL5 (right) from serum (diamonds) or plasma (squares) are shown on a logarithmic scale for 6 of the 51 patients examined in this study.

as well as increased tumor growth and microvessel density in vivo compared to vector-transfected controls [34]. Another recent study showed that androgen-insensitive DU145 and PC3 cells exposed to exogenous IL-8 demonstrated a rapid, time-dependent increase in cyclin D1 expression concordant with cellular proliferation [35]. Taken together, these studies suggested that IL-8 promotes androgen-independent PCa growth and progression, and may serve as a marker to distinguish between benign and malignant proliferative disease in the prostate.

Other reports have clearly identified the chemokine CXCL12 as a paracrine factor secreted by carcinoma-associated fibroblasts (CAFs). Allinen et al. demonstrated that CXCL12 was expressed and secreted at higher levels by invasive ductal breast carcinoma-associated stromal fibroblasts compared to normal-associated stromal fibroblasts (NAFs), and could promote the proliferation of cultured breast cancer epithelial cells [36]. Orimo et al. showed that CAFs extracted from invasive human breast carcinomas are more competent than non-transformed fibroblasts in enhancing tumor growth of breast cancer cells, and secrete increased levels of CXCL12 which both recruits endothelial progenitor cells into a tumor mass, thereby boosting tumor angiogenesis and enhances

tumor growth by direct paracrine stimulation via the CXCR4 receptor [37]. Studies utilizing co-cultured non-transformed human prostate-derived fibroblasts and the LNCaP PCa cell line clearly demonstrated that epithelial–stromal paracrine interactions enhanced the ability of xenografted LNCaP cells to grow as tumors in athymic mice [38]. Further work showed that prostatic CAFs stimulated tumor progression of immortalized non-tumorigenic epithelial cells both in vitro and in vivo [39]. Many studies have shown that chemokine–chemokine receptor interactions, particularly those between CXCL12 and CXCR4, stimulate chemotaxis in vitro and promote metastasis in vivo in prostate [40–43] breast [44–49] and lung [50] cancers. Taken together, these studies show that CXCR4/CXCL12-mediated signaling is involved in breast, small cell lung, and PCa cell proliferation and acquisition of an invasive phenotype in vitro and in vivo. Our finding, that serum CXCL12 levels are elevated in men with PCa, is consistent with these studies and with a role for this paracrine factor in prostate tumorigenesis.

A concern with the patient population utilized in these studies was that the presence of histological prostatic inflammation might confound the interpretation of data based on the measurement of serum chemokines, which are inflammatory mediators. Although none of the patients enrolled in this study complained of symptoms consistent with prostatitis or had sought medical treatment for prostatitis, almost one-third (16/51) of the study population exhibited histological evidence of acute (9/16, 56%), both acute and chronic (3/16, 19%), or chronic (4/16, 25%) inflammation in one or more needle biopsy specimens. Serum CXCL5 levels were progressively elevated in men with BPH concurrent with histological prostatitis ($P = 0.003$) or cancer ($P = 0.061$) compared to men without prostatic disease, though serum CXCL12 levels were unaffected by the presence or absence of histological prostatitis in the same patients. It is unclear, however, whether evidence of histological prostatitis, and, consequently, serum chemokine levels relative to histological prostatitis, are clinically relevant. Several studies have noted that prostatic inflammation is an extremely common histological finding in patients with BPH and/or cancer [9–12]. Hochreiter et al. also reported that the levels of two chemokines, IL-8 and CXCL5, were frequently elevated in the expressed prostatic secretions from men diagnosed with bacterial prostatitis, inflammatory chronic pelvic pain syndrome, and asymptomatic inflammatory prostatitis compared to normal controls. They hypothesized that, because these cytokines are direct mediators of leukocyte accumulation and activation at inflammatory sites, they may be responsible, in part, for the presence of inflammatory reactions in the prostate [51]. Taken together, these studies suggest a positive association between histological prostatitis or clinically significant prostatic inflammation and elevated serum or prostatic CXCL5 levels. They also suggest a potential association between prostatic inflammation and enlargement. Our findings, that serum CXCL5 levels were progressively elevated in men with BPH concurrent with histological prostatitis ($P = 0.003$) or cancer ($P = 0.061$) compared to men without prostatic disease, is an intriguing addition to the growing literature associating inflammation with BPH and cancer. They also point to the need to develop experimental models that provide the means to directly test the role of inflammation in the development of benign prostatic proliferative disease.

## CONCLUSIONS

Though preliminary, the results of the pilot and feasibility study reported here suggest that CXCL12 may provide a serum or plasma biomarker for the detection of PCa among men without BPH, while CXCL5 may provide the same among men with BPH. The use of such serum or plasma markers as adjuncts to serum PSA may facilitate decisions to perform diagnostic needle biopsy in the increasingly large patient population referred to Urology clinics who exhibit detectable serum PSA values of $<10$ ng/ml. Though dictated by the actual number of patient samples available at the time of study initiation, a clearly limiting factor of the study presented here is the relatively small sample size examined. Due to this limitation, these study results should be interpreted with caution pending their validation in a larger study population and, most importantly in a larger, multi-institutional study to thoroughly test the efficacy of these or other chemokines as serum or plasma biomarker adjuncts to serum PSA for the indication of diagnostic needle biopsy for the detection of PCa.

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