CCL11 (Eotaxin-1): A New Diagnostic Serum Marker for Prostate Cancer

Manisha Agarwal,[1] Chang He,[1] Javed Siddiqui,[2] John T. Wei,[1,2] and Jill A. Macoska[1]*

[1]Department of Urology, The University of Michigan School of Medicine, Ann Arbor, Michigan
[2]Center for Translational Pathology, The University of Michigan School of Medicine, Ann Arbor, Michigan

BACKGROUND. The recent recommendation of the U.S. Preventive Services Task Force against PSA-based screening for prostate cancer was based, in part, on the lack of demonstrated diagnostic utility of serum PSA values in the low, but detectable range to successfully predict prostate cancer. Though controversial, this recommendation reinforced the critical need to develop, validate, and determine the utility of other serum and/or urine transcript and protein markers as diagnostic markers for PCa. The studies described here were intended to determine whether inflammatory cytokines might augment serum PSA as a diagnostic marker for prostate cancer.
METHODS. Multiplex ELISA assays were performed to quantify CCL1, CCL2, CCL5, CCL8, CCL11, CCL17, CXCL1, CXCL5, CXCL8, CXCL10, CXCL12, and IL-6 protein levels in the serum of 272 men demonstrating serum PSA values of <10 ng/ml and undergoing a 12 core diagnostic needle biopsy for detection of prostate cancer. Logistic regression was used to identify the associations between specific chemokines and prostate cancer status adjusted for prostate volume, and baseline PSA.
RESULTS. Serum levels for CCL1 (I-309) were significantly elevated among all men with enlarged prostates ($P < 0.04$). Serum levels for CCL11 (Eotaxin-1) were significantly elevated among men with prostate cancer regardless of prostate size ($P < 0.01$). The remaining 10 cytokines examined in this study did not exhibit significant correlations with either prostate volume or cancer status.
CONCLUSIONS. Serum CCL11 values may provide a useful diagnostic tool to help distinguish between prostatic enlargement and prostate cancer among men demonstrating low, but detectable, serum PSA values. *Prostate 73: 573–581, 2013.* © 2012 Wiley Periodicals, Inc.

KEY WORDS: **prostate; cancer; serum; CCL11; Eotaxin-1; serum; biomarker**

INTRODUCTION

Detection rates for prostate cancer in men demonstrating total serum PSA 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 greater than six needle biopsy specimens [1,2]. These rates, however, are much lower for men demonstrating low total serum PSA (PSA) values of <10 ng/ml. For example, malignant glands are detected on needle biopsy for only ~30% of men whose PSA values were between 4 and 10 ng/ml, and tumor detection rates fall to 21–23% among men with detectable PSA values of <4 ng/ml [3–6]. These studies suggest that low (<10 ng/ml) PSA values are not diagnostic for PCa, and that factors other than cancer may contribute to the elevation in PSA in the serum, for example, prostatitis or BPH. A recent study showed that a smaller prostate volume is the strongest predictor of cancer detection in men exhibiting PSA levels in the 2.0–9.0 ng/ml range, suggesting that PSA is less useful for the prediction of cancer in men with concurrent BPH [7]. Several studies have shown that serum PSA

Grant sponsor: Early Detection Research Network; Grant sponsor: NIH/NCI; Grant number: U01 CA086402.

*Correspondence to: Jill A. Macoska, Department of Urology, The University of Michigan, 6217 Cancer Center, 1500 East Medical Center Drive, Ann Arbor, MI. E-mail: jcoska@umich.edu

Received 24 August 2012; Accepted 10 September 2012
DOI 10.1002/pros.22597
Published online 11 October 2012 in Wiley Online Library (wileyonlinelibrary.com).

© 2012 Wiley Periodicals, Inc.

values increase concomitantly with patient age in parallel with increased incidence of BPH [8–12].

Due, in part, to the lack of demonstrated screening utility of serum PSA values in the low, but detectable range (2.5–4.0 ng/ml), the U.S. Preventive Services Task Force (USPSTF) recently recommended against PSA-based screening for prostate cancer. This recommendation is controversial and has been criticized for not acknowledging the benefits of PSA screening, for example, that PSA screening is associated with a significant decline in prostate cancer-specific mortality in the U.S. over the past two decades [13,14]. Even so, the USPSTF recommendation does imply that low serum PSA values alone do not reliably predict clinically significant prostate tumors. Therefore, there is a critical need to develop, validate, and determine the utility of other serum and/or urine transcript and protein markers as diagnostic markers for PCa. Several markers identified and tested thus far, including prostate-specific membrane antigen (PSMA), Hepsin, a-methylacyl-coenzyme A racemase (AMACR), telomerase, the serine protease TMPRSS2, d-catenin, and a prostate-specific non-coding RNA called PCA3 (formerly called DD3), when used alone or in combination with serum PSA, demonstrate variable utilities as diagnostic or prognostic prostate cancer markers [15–18]. Taken together, these studies show that additional serum biomarkers would be very valuable to distinguish between prostatic diseases in men exhibiting low but detectable serum PSA.

We have previously shown that aging prostate stromal cells cultured in vitro secrete CXC-type chemokines, inflammatory proteins that act as potent growth factors that promote the proliferation of both non-transformed and transformed prostatic epithelial cells [19–24]. These observations potentially link stromally secreted inflammatory mediators with benign and malignant proliferative diseases of the prostate. We also reported preliminary studies suggesting that CXCL5 and CXCL12 might have diagnostic utility to distinguish PCa from BPH among men with low but detectable serum PSA [25]. We have now expanded these studies to test the efficacy of these and additional inflammatory CXC-, CC-, and IL-type chemokines and cytokines as serum biomarker for prostate cancer diagnosis and prognosis in men exhibiting low but detectable serum PSA values. The results of these studies show that CCL11 (aka Eotaxin), a CC-type chemokine not previously associated with prostatic disease, is detected at significantly ($P < 0.001$) higher levels in the serum of men with PCa than men with BPH or no evidence of prostatic disease. Moreover, serum CCL11 increases the diagnostic utility of serum PSA to detect prostate cancer, even among men exhibiting low but detectable serum PSA. These data suggest that serum CCL11 values may provide a useful diagnostic tool to help distinguish between BPH and PCa among men demonstrating low, but detectable, serum PSA values.

MATERIALS AND 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 prostate specific antigen (PSA), abnormal digital rectal exam (DRE), high grade prostatic intraepithelial neoplasia (HGPIN), or atypical small acinar proliferation (ASAP) on prior biopsy, age, positive family history of prostate cancer, and race/ethnicity. The Prostate Cancer Prevention Trial Prostate Cancer Risk Calculator (PCPTRC) was calculated for each patient using this data [26]. 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. The final study population of 272 patients was selected for those exhibiting pre-biopsy total serum PSA values of <10 ng/ml (determined using the Abbott AxSYM polyclonal–monoclonal immunoassay [Abbott Diagnostics, Abbott Park, Ill]), and that permitted examination of equivalent cases of high volume disease within both the biopsy-negative and -positive cases (Table I).

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 volume (PV) 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 was gathered during a standard TRUS examination performed using a 7.5 MHz

TABLE I. Patient Demographic, Clinical, and Histopathological Data

Category	Sub-category	All patients	No disease	BPH	PCa-BPH	PCa + BPH	*P*-value	Method
Total number		272	68	71	67	66		
Median prostate volume (range)		40.7 (16.2–279.0)	33 (18–40)	60.7 (40.2–132.0)	32.8 (16.2–40.0)	57.5 (40.7–279.0)	<0.0001	Kruskal–Wallis
Mean pre-biopsy PSA, pg/ml (SE)		5.0 (0.1)	3.9 (0.3)	5.4 (0.3)	5.2 (0.3)	5.5 (0.2)	0.0001	One-way ANOVA
Ethnicity	Caucasian (%)	229	54 (23.6)	60 (26.2)	57 (24.9)	58 (25.3)	0.1	Chi-squared
	African American (%)	19	4 (21.1)	3 (15.8)	6 (31.6)	6 (31.6)		
	Asian (%)	18	9 (50.0)	6 (33.3)	2 (11.1)	1 (5.6)		
	Other/ND	6						
Median Gleason sum (range)		7 (6–9)	ND	ND	7 (6–9)	7 (6–9)	0.11	Wilcoxon rank sum test
Mean age at surgery (SE)		61.4 (0.5)	58.4 (1.1)	61.2 (0.9)	60.9 (1.0)	65.0 (0.9)	0.0002	One-way ANOVA
Family history	Yes (%)	79	16 (20.3)	18 (22.8)	25 (31.7)	20 (25.3)	0.27	Chi-squared
	No (%)	191	52 (27.2)	52 (27.2)	41 (21.5)	46 (24.1)		
	ND	2						
DRE results	Normal (%)	59	25 (42.4)	7 (11.9)	21 (35.6)	6 (10.2)	<0.0001	Fisher's exact test
	Enlarged, benign (%)	152	25 (16.5)	51 (33.6)	29 (19.1)	47 (31.0)		
	Enlarged/asymmetric (%)	10	0 (0)	4 (40.0)	3 (30.0)	3 (30.0)		
	Abnormal, cancer suspicious (%)	51	18 (35.3)	9 (17.7)	14 (27.5)	10 (19.6)		

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 $= \pi/6(\text{TRxAPxSI})$.

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) for 1 week prior to biopsy to minimize bleeding. For all patients, blood was drawn into 10 cm^3 venous blood collection tubes (#366430, Beckton, Dickinson, Franklin Lakes, NJ), which were placed on ice and processed within 12 hr. The blood was transferred into 15 ml tubes, centrifuged at 2,500 rpm for 10 min, and stored in 200 μl aliquots in 0.5 ml cryovials (Sarstedt, Newton, NC) 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 μl 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 [27]. 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 (high grade prostatic intraepithelial neoplasia)/ASAP (atypical small acinar proliferation), 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 evaluation of the entire tumor.

Definitions of Prostatic Disease Status and Study Groups

Disease status was carefully defined in the study group, as delineated below and detailed in Table I.

No disease. *Criteria*: No finding of cancer on prostate biopsy. Prostate volume <40 g on TRUS. Prostate biopsy specimens evaluated as normal benign. PSA values <10 ng/ml. This comprised 68/272 (25%) of the total patient population examined in this study.

BPH. *Criteria*: Evidence for enlarged prostate, defined as ≥40 g, roughly equivalent to measures described as high-volume and consistent with BPH in the literature [7]. Men with enlarged prostates that were biopsy-negative for prostate cancer. These patients comprised 71/272 (26%) of the total patient population examined in this study. Although the American Urological Association Symptom Index (AUASI) was recorded for all patients, it was not used to define BPH.

BPH concurrent with PCa. *Criteria*: Men with enlarged prostates as defined above and biopsy-positive for prostate cancer. Cancer was assessed from a histological diagnosis of malignant glands in one or more diagnostic needle biopsies. Five board-certified pathologists were involved in the histologic diagnoses of the prostate biopsies. These patients comprised 66/272 (24%) of the total patient population examined in this study.

PCa in the absence of BPH. *Criteria*: Men with prostates <40 g and biopsy-positive for prostate cancer. These patients comprised 67/272 (25%) of the total patient population examined in this study.

Multiplex ELISA Assays

Multiplex ELISA assays were performed by QUANSYS Biosciences (Logan, UT). The arrays were modified from the QUANSYS human cytokine array to include capture antibodies for CCL1 (I-309), CCL2 (MCP-1), CCL5 (RANTES), CCL8 (MCP-2), CCL11 (Eotaxin), CCL17 (TARC), CXCL1 (GRO-α), CXCL5 (ENA-78), CXCL8 (IL-8), CXCL10 (IP-10), CXCL12 (SDF-1), and IL-6 spotted in a 3 × 4 grid into the bottom of each well of a 96-well-plate. Each spotted well received 30 μl serum, the plate incubated for 1 hr, wells washed, and 30 μl of a pooled detection antibody cocktail was added. The plate was incubated for 1 hr, washed, and streptavidin bound to horse radish peroxidase (HRP) was added. Chemiluminscence was visualized and imaged using a Q-View Imager and signals quantitated against standard curves generated from known standards included on the plates using Q-View Software. Each assay was performed in triplicate and the resulting data was averaged. For the assays utilized on the modified human cytokine array using undiluted samples, the coefficient of variation (CV) between assays within a triplicate set varied for each assay, with assays for CCL11, CCL8, and CXCL5 demonstrating the lowest (<5%) CVs, followed by CXCL8, CXCL10, CCL2, CCL5, and CCL17 (5–10%), CXCL1 and CCL1 (10–15%), and IL-6 (20%; Table II).

Statistical Analysis

The bivariate relationship of circulating cytokine/chemokine levels and disease status was tested in this patient population using the non-parametric Wilcoxon rank-sum test, as we have done previously with a similar but smaller dataset [25]. Separate tests were performed for each definition of disease in pairwise fashion. Multivariable analyses receiver operator curve (ROC) analyses were pursued to examine sensitivity and specificity for individual chemokines as predictors of disease and to calculate the area under the curve (AUC) and confidence intervals (CIs). The logistic regression was used to identify the statistic association between specific chemokines and prostate disease status adjusted for prostate volume, and baseline PSA. Backward model building procedure were used to determine the most parsimonious models for various prostate

TABLE II. Percent Coefficient of Variation (% CV) for Individual Assays

% CV	CCL11 (Eotaxin-1)[a]	CXCL1 (GRO-a)	CCL1 (I-309)	CXCL8 (IL-8)	CXCL10 (IP-10)	CCL2 (MCP-1)	CCL8 (MCP-2)	CCL5 (RANTES)	CCL17 (TARC)	IL-6	CXCL5 (ENA-78)
≤5%	5.0						4.9				4.6
>5–10%				7.7	5.6	5.3		6.1	8.0		
>10–15%		14.5	13.7								
>15–20%										20	

[a]Former nomenclature is shown in parentheses.

disease statuses. All tests were performed using SAS v 9.2 at the 5% significance level.

RESULTS

As shown in Table I, the four patient groups included in this study—men with no prostatic disease, with BPH, with PCa in the absence of BPH, or with PCa concurrent with BPH—differed significantly for prostate size, pre-biopsy PSA, age at surgery, DRE results, and presence or absence of malignant glands in the prostate. Men with concurrent PCa and BPH were significantly older than men with no disease, BPH alone, or PCa without BPH (Table I). Men with no disease demonstrated significantly lower pre-biopsy serum PSA values than men in the other three patient groups (Table I).

The levels of 12 proteins—CCL1 (I-309), CCL2 (MCP-1), CCL5 (RANTES), CCL8 (MCP-2), CCL11 (Eotaxin-1), CCL17 (TARC), CXCL1 (GRO-α), CXCL5 (ENA-78), CXCL8 (IL-8), CXCL10 (IP-10), CXCL12 (SDF-1), and IL-6 were measured by multiplex ELISA in serum from 272 patients who presented with low but detectable serum PSA levels of <10 ng/ml. All of these proteins were robustly, though differentially, detectable in the serum of these patients (Table III) with the exception of CXCL12. The assays for CXCL5 and CXCL12 were custom-designed by Quansys Biosciences specifically for this study. Intra-assay precision tests performed as part of the development of these assays showed that percent recovery (detection) of known levels of recombinant CXCL5 was 93%, well within the desired range of 80–120% defined by Quansys Biosciences. However, the percent recovery of known levels of recombinant CXCL12 was only 63%, much lower than desired. Of the 272 patient serum samples tested, CXCL12 was detected in only 12 samples. The only known receptor for CXCL12, CXCR4, is present on platelets in serum, which can bind CXCL12 with high affinity, making CXCL12 unavailable for binding to the capture antibody in an ELISA assay [28–31]. Thus, serum levels of CXCL12 could not be reliably measured in this study.

The protein with the highest measured serum level in this study was RANTES (CCL5), which averaged >22,000 pg/ml across all four patient groups (Table III). Conversely, the protein with the lowest measured serum level was IL-6, which averaged only 3.63–6.65 pg/ml across all four patient groups (Table III). Neither these nor CCL2, CCL8, CCL17, CXCL1, CXCL5, CXCL8, or CXCL10 demonstrated significantly different serum levels across the four patient groups included in this study (Table III).

Two serum proteins, CCL1 (I-309) and CCL11 (Eotaxin-1), were differentially represented in the serum of the patient groups tested in this study. Serum levels for CCL1 differed significantly between the four patient groups ($P < 0.04$). Logistic regression showed that serum CCL1 levels were significantly elevated ($P = 0.017$) among all men with enlarged prostates, defined as TRUS PV ≥40 g (Table III). Serum levels for CCL11 also differed significantly between the four patient groups (CCL11) ($P < 0.01$) and were specifically elevated among men with prostate cancer regardless of prostate size. Moreover, serum CCL11 levels for men biopsy-negative for prostate cancer (no disease or BPH alone) averaged 101 pg/ml, whereas those for men biopsy-positive for prostate cancer (PCa + BPH and PCa-BPH) were significantly ($P < 0.001$) higher and averaged 128 pg/ml (Table IV). Thus, CCL11 successfully identified prostate tumors in men independent of prostate volume.

TABLE III. Serum Chemokine and Cytokine Levels

	No disease (N = 68)		BPH (N = 71)		PCa-BPH (N = 67)		PCa + BPH (N = 66)		
Serum protein	Mean	SE[a]	Mean	SE	Mean	SE	Mean	SE	P-value
CCL1	6.37	0.82	8.38	0.79	7.33	0.85	9.57	0.87	0.04
CCL2	198.29	9.95	209.63	9.7	216.95	9.97	217.02	10.03	0.50
CCL5	22565.00	1706.48	22122.94	1657.89	24684.02	1709.85	25972.93	1726.05	0.34
CCL8	27.26	1.51	26.01	1.47	27.92	1.51	24.73	1.53	0.46
CCL11	99.19	8.21	104.2	7.95	123.24	8.23	133.69	8.24	0.01
CCL17	191.33	15.59	189.14	15.09	198.01	15.63	200.6	15.71	0.95
CXCL1	16.98	3.09	13.98	3.5	9.68	3.06	16.95	3.36	0.33
CXCL5	4015.22	528.29	3618.64	605.58	4546.48	576.63	3633.13	579.97	0.64
CXCL8	6.66	0.48	7.37	0.46	6.97	0.48	7.61	0.48	0.51
CXCL10	76.77	8.94	65.16	8.65	62.44	8.96	70.32	9.11	0.68
IL-6	5.12	1.24	3.78	1.15	3.63	1.28	6.65	1.27	0.28

All values are pg/ml.
[a]SE, standard error of the mean.

TABLE IV. Serum Chemokine and Cytokine Levels in Patients Harboring Non-cancerous or Cancerous Prostates

	No cancer	Cancer	*P*-value
CCL11	101.36 ± 5.68	128.16 ± 5.80	0.001
CCL1	7.33 ± 0.58	8.44 ± 0.61	0.190
CCL2	204.08 ± 6.93	216.99 ± 7.05	0.190
CCL5	22339.28 ± 1185.27	25323.63 ± 1210.77	0.080
CCL8	26.62 ± 1.06	26.35 ± 1.08	0.860
CCL17	190.21 ± 10.80	199.29 ± 11.04	0.560
CXCL1	15.37 ± 2.34	13.17 ± 2.30	0.500
CXCL5	3845.26 ± 397.93	4081.79 ± 408.84	0.680
CXCL8	7.03 ± 0.33	7.29 ± 0.34	0.580
CXCL10	70.84 ± 6.21	66.32 ± 6.38	0.610
IL-6	4.44 ± 0.85	5.13 ± 0.90	0.580

All values are pg/ml. Data shown is mean ± SD.

We next compared the ability of serum CCL11 to serum PSA to predict prostate tumors. The AUC calculated in this patient set using logistic regression and ROC analyses was 0.57 (CI 0.51–0.66) for PSA and 0.56 (CI 0.48–0.64) for CCL11. When combined, CCL11 + PSA increased the AUC to 0.62 (CI 0.54–0.69), indicating that CCL11 + PSA serum levels better predicted the presence of prostate cancer than either PSA or CCL11 alone (Fig. 1). However, this difference between the predictive values of CCL11 and PSA ($P = 0.65$) and between CCL11 + PSA compared to that of PSA alone ($P = 0.18$) did not reach statistical significance ($P = 0.18$). Similarly, the ability of CCL11 to predict prostate cancer was compared to that of The Prostate Cancer Prevention Trial Prostate Cancer Risk Calculator (PCPTRC) [26]. The AUC calculated in this patient set using logistic regression and ROC analyses was 0.60 (CI 0.53–0.68) for the PCPTRC. When combined, CCL11 + PCPTRC increased the AUC to 0.64 (CI 0.56–0.71), indicating that CCL11 serum levels combined with PCPTRC risk analysis better predicted the presence of prostate cancer than either CCL11 or PCPTRC alone (Fig. 1). However, this difference between the predictive values of CCL11 and PCPTRC ($P = 0.39$) and between CCL11 + PCPTRC compared to that of PCPTRC alone ($P = 0.19$) did not reach statistical significance. Thus, although CCL11 successfully identified prostate tumors in men independent of prostate volume, the predictive value of CCL11 was not significantly different from that of either PSA or PCPTRC.

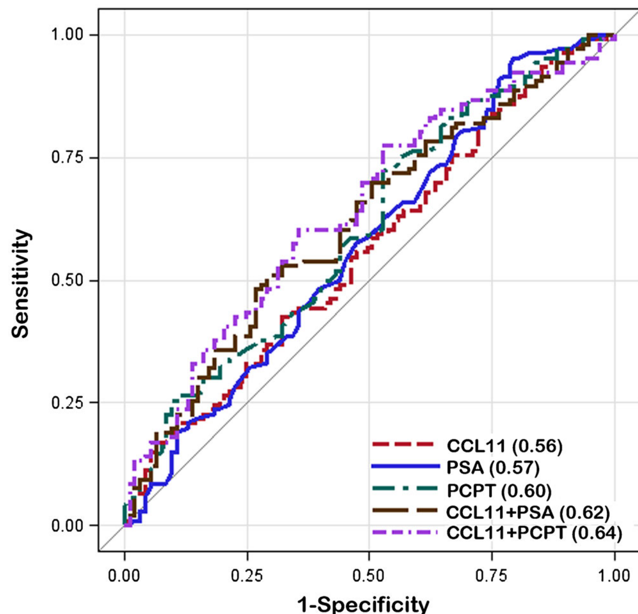

Fig. 1. Serum CCL11 levels increase the diagnostic utility of serum PSA and PCPTRC risk analysis to predict prostate cancer. Receiver-operator curves demonstrating the ability of serum PSA, PCPTRC (PCTP), and serum CCL11, alone or in combination, to predict prostate cancer on patients with serum PSA <10 ng/ml (N = 272). The area under the curve (AUC) for each measure is indicated in parentheses.

DISCUSSION

The studies described here were intended to determine whether cytokine and/or chemokine serum proteins could be identified that might augment or replace serum PSA as a diagnostic marker for prostate cancer.

Although 12 markers were initially investigated as potential prostate cancer biomarkers, only two—CCL1 and CCL11—demonstrated utility in this regard. One of them, CCL1, actually demonstrated elevated levels in association with BPH rather than PCa. CCL1 is a small (15–18 kdal) secreted glycoprotein and is the sole ligand for the G-protein coupled receptor, CCR8 [32]. An earlier study had described elevated levels of CCL1 in seminal plasmas from patients

with BPH [33]. Although high levels of CCL1 protein have now been identified in both the serum and seminal fluid of men with enlarged prostates, the cellular source for CCL1 expression is likely activated T-cells [34] in the prostate tissue microenvironment. The CCR8 receptor is expressed on a variety of cell types including monocytes and endothelial cells and is selectively up-regulated upon activation of T-helper-2 (Th2) cells [35]. Inflammatory infiltrates are very commonly observed in BPH specimens and comprise 70% T lymphocytes, 15% B cells, and 15% macrophages as well as mast cells [36–38]. The T lymphocyte population in particular expands enormously in BPH/LUTS, increasing from a mean of 7 cells/mm^2 in the normal prostate to a mean of 195 cells/mm^2 in fully developed BPH [38]. Therefore, it is not surprising that a chemokine largely secreted by activated T-cells may be found at significantly elevated levels in the serum of men with enlarged prostates. Other studies have demonstrated that T-lymphocytes promoted the proliferation of both transformed and non-transformed prostate epithelial cell lines in vitro [39], suggesting that high serum levels of CCL1 may signify the presence of large T-lymphocyte populations in the prostate that, in turn, promote prostate tissue proliferation and prostatic enlargement.

The second serum protein biomarker identified in this study, CCL11 (Eotaxin-1), is a potent chemotactic factor for eosinophils [40]. CCL11 is a ligand for the G-protein coupled receptor, CCR3, which is expressed on eosinophils, basophils, and Th2 helper T-cells [41–43]. Very little is known regarding potential role(s) for CCL11 in prostate pathology. A recent study reported up-regulation of CCL11 transcript and those of many other CXC- and CC-type chemokines in a mouse model of experimental autoimmune prostatitis [44]. Another study reported up-regulation of CCL11 in the prostates of male Wistar rats chronically exposed to estradiol [45]. Although there are no reports examining serum CCL11 levels in men with benign or malignant prostatic disease, several studies report that elevated serum CCL11 levels are diagnostic or prognostic for other human cancers. For example, a recent study showed that elevated serum CCL11 and osteopontin levels were significantly ($P = 0.016$ and 0.021, respectively) associated with disease progression in patients with head and neck small cell carcinomas (HNSCCs) receiving induction therapy on a Phase II trial of carboplatin, paclitaxel, and cetuximab [46]. Another study demonstrated that CCR3 expression correlated with high grade renal cell carcinomas (RCCs) [47]. Moreover, this study showed that A-498 renal cancer cells demonstrated a robust proliferative response to treatment with CCL11 in vitro [47]. In a mouse model of skeletal breast cancer metastasis, vascular endothelial growth factor (VEGF) and CCL11 levels were significantly higher in the femurs of cancer cell-inoculated compared to sham-inoculated mice [48]. This study speculated that the pro-angiogenic functions of VEGF and CCL11 may enable metastatic cancer cells to colonize and thrive in the bone environment [48]. A proteomics approach recently identified high plasma levels of CCL11 as predictive for high risk neuroblastoma, and included CCL11 levels within a 7-protein classifier set of plasma proteins potentially useful for the detection of relapse prior to the development of clinically evident disease [49]. Taken together, these studies are consistent with the use of CCL11 plasma or serum levels as diagnostic or prognostic cancer markers.

Lastly, the studies reported here also showed that the combined use of serum CCL11 levels with serum PSA or PCPTRC risk analysis increases the diagnostic utility of all of these measures to detect prostate cancer, even among men exhibiting low but detectable serum PSA. Recent studies have demonstrated the utility of 'adding' PCA3 and TMPRSS2/ERG urine transcript levels to serum PSA levels or PCPTRC risk analysis to improve prostate cancer diagnosis [50]. Clearly, there is a need to continue to identify and validate serum, plasma, and urine biomarkers that can be used in combination to identify men at high risk for harboring clinically significant prostate tumors. In this way, initial screening tools can be developed and tested that enable clinicians to make sound recommendations for the use of secondary diagnostic tools, such as ultrasound and needle biopsy, to accurately detect clinically significant prostate tumors.

ACKNOWLEDGMENTS

This work was supported by an Early Detection Research Network (EDRN) NIH/NCI award U01 CA086402 (J.A.M.).

REFERENCES

1. Luciani LG, De Giorgi G, Valotto C, Zanin M, Bierti S, Zattoni F. Role of transperineal six-core prostate biopsy in patients with prostate-specific antigen level greater than 10 ng/mL and abnormal digital rectal examination findings. Urology 2006; 67(3):555–558.
2. Inahara M, Suzuki H, Kojima S, Komiya A, Fukasawa S, Imamoto T, Naya Y, Ichikawa T. Improved prostate cancer detection using systematic 14-core biopsy for large prostate glands with normal digital rectal examination findings. Urology 2006;68(4):815–819.
3. Kravchick S, Peled R, Dorfman D, Agulansky L, Ben-Dor D, Cytron S. Predictive criteria for prostate cancer detection in men with serum PSA concentration of 2.0 to 4.0 ng/mL. Urology 2005;66(3):542–546.

4. Pelzer AE, Tewari A, Bektic J, Berger AP, Frauscher F, Bartsch G, et al. Detection rates and biologic significance of prostate cancer with PSA less than 4.0 ng/mL: Observation and clinical implications from Tyrol screening project. Urology 2005;66(5): 1029–1033.
5. Gilbert SM, Cavallo CB, Kahane H, Lowe FC. Evidence suggesting PSA cutpoint of 2.5 ng/mL for prompting prostate biopsy: review of 36,316 biopsies. Urology 2005;65(3):549–553.
6. Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360(13): 1320–1328.
7. Al-Azab R, Toi A, Lockwood G, Kulkarni GS, Fleshner N. Prostate volume is strongest predictor of cancer diagnosis at transrectal ultrasound-guided prostate biopsy with prostate-specific antigen values between 2.0 and 9.0 ng/mL. Urology 2007; 69(1):103–107.
8. Roehrborn CG, Boyle P, Gould AL, Waldstreicher J. Serum prostate-specific antigen as a predictor of prostate volume in men with benign prostatic hyperplasia. Urology 1999;53(3): 581–589.
9. Wright EJ, Fang J, Metter EJ, Partin AW, Landis P, Chan DW, et al. Prostate specific antigen predicts the long-term risk of prostate enlargement: Results from the Baltimore longitudinal study of aging. J Urol 2002;167(6):2484–2487; discussion 7–8.
10. Berger AP, Cheli C, Levine R, Klocker H, Bartsch G, Horninger W. Impact of age on complexed PSA levels in men with total PSA levels of up to 20 ng/mL. Urology 2003;62(5):840–844.
11. Berger AP, Deibl M, Strasak A, Bektic J, Pelzer AE, Klocker H, et al. Large-scale study of clinical impact of PSA velocity: Long-term PSA kinetics as method of differentiating men with from those without prostate cancer. Urology 2007;69(1):134–138.
12. Pinsky PF, Kramer BS, Crawford ED, Grubb RL, Urban DA, Andriole GL, et al. Prostate volume and prostate-specific antigen levels in men enrolled in a large screening trial. Urology 2006;68(2):352–356.
13. Collin SM, Martin RM, Metcalfe C, Gunnell D, Albertsen PC, Neal D, et al. Prostate-cancer mortality in the USA and UK in 1975–2004: An ecological study. Lancet Oncol 2008;9(5):445–452; PMCID: 2760747.
14. Miller DC, Hollenbeck BK. Missing the mark on prostate-specific antigen screening. JAMA 2011;306(24):2719–2720.
15. Cazares LH, Drake RR, Esquela-Kirscher A, Lance RS, Semmes OJ, Troyer DA. Molecular pathology of prostate cancer. Cancer Biomark 2010;9(1–6):441–459.
16. Martin NE, Mucci LA, Loda M, Depinho RA. Prognostic determinants in prostate cancer. Cancer J 2011;17(6):429–437; PMCID: 3240856.
17. Netto GJ, Cheng L. Emerging critical role of molecular testing in diagnostic genitourinary pathology. Arch Pathol Lab Med 2012;136(4):372–390.
18. Salami SS, Schmidt F, Laxman B, Regan MM, Rickman DS, Scherr D, Bueti G, Siddiqui J, Tomlins SA, Wei JT, Chinnaiyan AM, Rubin MA, Sanda MG. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. Urol Oncol Available online 19 May 2011, ISSN 1078-1439; PMCID: 3210917.
19. Begley L, Monteleon C, Shah RB, Macdonald JW, Macoska JA. CXCL12 overexpression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. Aging Cell 2005;4(6):291–298.
20. Begley LA, Kasina S, MacDonald J, Macoska JA. The inflammatory microenvironment of the aging prostate facilitates cellular proliferation and hypertrophy. Cytokine 2008;43(2):194–199; PMCID: 2538565.
21. Begley LA, Kasina S, Mehra R, Adsule S, Admon AJ, Lonigro RJ, et al. CXCL5 promotes prostate cancer progression. Neoplasia 2008;10(3):244–254; PMCID: 2262133.
22. Begley LA, MacDonald JW, Day ML, Macoska JA. CXCL12 activates a robust transcriptional response in human prostate epithelial cells. J Biol Chem 2007;282(37):26767–26774.
23. Kasina S, Macoska JA. The CXCL12/CXCR4 axis promotes ligand-independent activation of the androgen receptor. Mol Cell Endocrinol 2012;351(2):249–263; PMCID: 3307100.
24. Kasina S, Scherle PA, Hall CL, Macoska JA. ADAM-mediated amphiregulin shedding and EGFR transactivation. Cell Prolif 2009;42(6):799–812; PMCID: 3167473.
25. Macoska JA, Begley LA, Dunn RL, Siddiqui J, Wei JT, Sarma AV. Pilot and feasibility study of serum chemokines as markers to distinguish prostatic disease in men with low total serum PSA. Prostate 2008;68(4):442–452.
26. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, et al. Assessing prostate cancer risk: Results from the prostate cancer prevention trial. J Natl Cancer Inst 2006; 98(8):529–534.
27. Siu W, Dunn RL, Shah RB, Wei JT. Use of extended pattern technique for initial prostate biopsy. J Urol 2005;174(2):505–509.
28. Heveker N, Montes M, Germeroth L, Amara A, Trautmann A, Alizon M, et al. Dissociation of the signalling and antiviral properties of SDF-1-derived small peptides. Curr Biol 1998; 8(7):369–376.
29. Kowalska MA, Ratajczak J, Hoxie J, Brass LF, Gewirtz A, Poncz M, et al. Megakaryocyte precursors, megakaryocytes and platelets express the HIV co-receptor CXCR4 on their surface: Determination of response to stromal-derived factor-1 by megakaryocytes and platelets. Br J Haematol 1999;104(2):220–229.
30. Wang JF, Liu ZY, Groopman JE. The alpha-chemokine receptor CXCR4 is expressed on the megakaryocytic lineage from progenitor to platelets and modulates migration and adhesion. Blood 1998;92(3):756–764.
31. Abi-Younes S, Sauty A, Mach F, Sukhova GK, Libby P, Luster AD. The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques. Circ Res 2000;86(2):131–138.
32. Roos RS, Loetscher M, Legler DF, Clark-Lewis I, Baggiolini M, Moser B. Identification of CCR8, the receptor for the human CC chemokine I-309. J Biol Chem 1997;272(28):17251–17254.
33. Penna G, Mondaini N, Amuchastegui S, Degli Innocenti S, Carini M, Giubilei G, et al. Seminal plasma cytokines and chemokines in prostate inflammation: Interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. Eur Urol 2007;51(2):524–533.
34. Miller MD, Krangel MS. The human cytokine I-309 is a monocyte chemoattractant. Proc Natl Acad Sci USA 1992;89(7):2950–2954; PMCID: 48781.
35. Zingoni A, Soto H, Hedrick JA, Stoppacciaro A, Storlazzi CT, Sinigaglia F, et al. The chemokine receptor CCR8 is preferentially expressed in Th2 but not Th1 cells. J Immunol 1998; 161(2):547–551.
36. Theyer G, Kramer G, Assmann I, Sherwood E, Preinfalk W, Marberger M, et al. Phenotypic characterization of infiltrating

leukocytes in benign prostatic hyperplasia. Lab Invest 1992; 66(1):96–107.

37. Steiner GE, Stix U, Handisurya A, Willheim M, Haitel A, Reithmayr F, et al. Cytokine expression pattern in benign prostatic hyperplasia infiltrating T cells and impact of lymphocytic infiltration on cytokine mRNA profile in prostatic tissue. Lab Invest 2003;83(8):1131–1146.
38. Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? Eur Urol 2007;51(5):1202–1216.
39. McDowell KL, Begley LA, Mor-Vaknin N, Markovitz DM, Macoska JA. Leukocytic promotion of prostate cellular proliferation. Prostate 2010;70(4):377–389; PMCID: 3167472.
40. Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P, Luster AD. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. Nat Med 1996;2(4):449–456.
41. Gerber BO, Zanni MP, Uguccioni M, Loetscher M, Mackay CR, Pichler WJ, et al. Functional expression of the eotaxin receptor CCR3 in T lymphocytes co-localizing with eosinophils. Curr Biol 1997;7(11):836–843.
42. Uguccioni M, Mackay CR, Ochensberger B, Loetscher P, Rhis S, LaRosa GJ, et al. High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines. J Clin Invest 1997;100(5):1137–1143; PMCID: 508288.
43. Sallusto F, Mackay CR, Lanzavecchia A. Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 1997;277(5334):2005–2007.
44. Quick ML, Mukherjee S, Rudick CN, Done JD, Schaeffer AJ, Thumbikat P. CCL2 and CCL3 are essential mediators of pelvic pain in experimental autoimmune prostatitis. Am J Physiol Regul Integr Comp Physiol 2012;303(6):R580–R589.
45. Harris MT, Feldberg RS, Lau KM, Lazarus NH, Cochrane DE. Expression of proinflammatory genes during estrogen-induced inflammation of the rat prostate. Prostate 2000;44(1):19–25.
46. Byers LA, Holsinger FC, Kies MS, William WN, El-Naggar AK, Lee JJ, et al. Serum signature of hypoxia-regulated factors is associated with progression after induction therapy in head and neck squamous cell cancer. Mol Cancer Ther 2010;9(6): 1755–1763; PMCID: 2913168.
47. Johrer K, Zelle-Rieser C, Perathoner A, Moser P, Hager M, Ramoner R, et al. Up-regulation of functional chemokine receptor CCR3 in human renal cell carcinoma. Clin Cancer Res 2005; 11(7):2459–2465.
48. Sosnoski DM, Krishnan V, Kraemer WJ, Dunn-Lewis C, Mastro AM. Changes in cytokines of the bone microenvironment during breast cancer metastasis. Int J Breast Cancer 2012;2012: Article ID 160265, 9 pages, PMCID: 3270522.
49. Egler RA, Li Y, Dang TA, Peters TL, Leung E, Huang S, et al. An integrated proteomic approach to identifying circulating biomarkers in high-risk neuroblastoma and their potential in relapse monitoring. Proteomic Clin Appl 2011;5(9–10):532–541.
50. Tomlins SA, Aubin SM, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2: ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. Sci Transl Med 2011;3(94):94ra72; PMCID: 3245713.