Original Article

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Serum CCL11 Levels in Benign Prostatic Hyperplasia and Prostate Cancer

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Purpose: CC-chemokine ligand 11 (CCL11; eotaxin-1), an eosinophil chemoattractant chemokine, has been proposed as a serum marker for prostate cancer (PCa) by two research groups. We investigated the usefulness of CCL11 in diagnosing prostatic diseases, such as benign prostatic hyperplasia (BPH) and PCa.
Materials and Methods: CCL11 was measured in the sera of 139 men with BPH, 44 men with PCa, and 45 control men attending an outpatient health-screening clinic. A commercial enzyme-linked immunosorbent assay kit was used to measure CCL11.
Results: CCL11 concentrations were significantly higher in men with BPH and PCa than in normal men (72.9±3.15 and 80.0±4.91 pg/ml vs. 57.6±8.24). In addition, a receiver operating characteristic (ROC) analysis of serum CCL11 levels showed that the areas under the ROC curves were 0.661 (p=0.001) and 0.654 (p=0.012) for BPH and PCa, respectively, compared with normal men.
Conclusions: CCL11 may be helpful in diagnosing prostatic diseases, such as BPH and PCa.

Keywords: Chemokine CCL11; Prostatic hyperplasia; Prostatic neoplasms

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INTRODUCTION

Eotaxin-1, also known as CC-chemokine ligand 11 (CCL11), is a protein expressed in various organs, such as the heart, central nervous system, and cerebrospinal fluid. Moreover, it is involved in immunoregulatory and inflammatory processes and acts as a selective eosinophil chemo-attractant [1].

Chemokines are a large family of structurally homologous cytokines that are secreted by a variety of cells, including structural cells and leukocytes of the immune system. They bind to specific 7-transmembrane G protein-coupled receptors, initiating a variety of downstream signals, notably ones that modulate polymerization of the actin cytoskeleton, ultimately driving cellular motility. Excessive or inappropriate release of chemokines is observed in many inflammatory diseases [2].

Agarwal et al. [3] and Heidegger et al. [4] have both reported that serum CCL11 may be a new diagnostic marker for prostate cancer (PCa). However, there were conflicting results; for PCa, one of them reported increased CCL11, while the other reported decreased CCL11. Such difference is likely due to different measurement methods.

In order to examine the possibility of using CCL11 as a diagnostic marker for prostatic diseases, the CCL11 levels were measured by enzyme-linked immunosorbent assay (ELISA) in individuals with benign prostatic hyperplasia (BPH) and PCa and compared with the levels in normal men.

MATERIALS AND METHODS

1. Patient Population and Specimen Collection

Between October 2013 and November 2014, a total of 183 men undergoing treatment in the Department of Urology at Hanyang University Seoul Hospital and Guri Hospital were enrolled in this study. Among them, there were 139 men with BPH (mean age, 64.0±0.07 years) and 44 men with PCa (mean age, 73.3±0.18 years).

The inclusion criteria for BPH were as follows: (1) A score of 8 or more according to the International Prostate Symptom Scores; (2) <4 ng/ml of prostate-specific antigen (PSA); (3) >20 ml of prostate volume (measured by transrectal ultrasonography; TRUS); and (4) >4 ng/ml of PSA and no evidence of malignant tumor on TRUS biopsy. Moreover, PCa patients were only selected if they had undergone a radiologic evaluation to determine their TNM clinical stage. A radiologic evaluation was carried out via chest computed tomography (CT), abdominal CT, prostate magnetic resonance imaging, and whole body bone scan.

The sera were obtained from 139 patients with BPH, 44 with PCa, and 45 male volunteers (mean age, 39.1±1.26 years) who visited Hanyang University International Hospital Health Promotion Center without any history of prostatic disease. Blood samples of 7 ml were collected, and plasma was stored at -70°C.

This study was approved by the local Institutional Review Board of Hanyang University, and written informed consent was obtained from all participants (IRB no. HYUH 2013-04-028-006).

2. CCL11 Measurement

The levels of CCL11 were determined with a human CCL11 ELISA kit (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. For the ELISA experiments, 96-well ELISA plates were coated with 5 μg/ml of diluted capture antibody (2 μg/ml) and incubated overnight at room temperature. The plates were washed thrice with phosphate-buffered saline (PBS)-Tween 20 (0.05% Tween 20 in PBS [pH 7.0]) followed by the addition of 200 μl/well of blocking buffer (reagent diluent). After incubation at room temperature for 1 h, the plates were again washed 3× with PBS-Tween 20, and 80 μl of serum was added to each well. After incubation for 2 h at room temperature, the plates were washed thirce with PBS-Tween 20. Following the incubation with 100 μl/well of detection antibody (0.2 μg/ml) for 2 h at room temperature, the plates were washed 3× with PBS-Tween 20. Streptavidin-HRP (1:200) was added, and the plates were incubated at room temperature for 20 min and washed 3× with PBS-Tween 20. The color was developed by adding 100 μl/well of substrate solution (TMB Microwell Peroxidase Substrate System; KPL, Gaithersburg, MD, USA) at room temperature for 20 min. The absorbance was measured with an ELISA reader (Bio-Rad, Hercules, CA, USA) at 450 nm after a 20 min incubation with stop solution (2.5 M H2SO4).

3. Statistical Analysis

IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA) was used for statistical analysis. Differences between the two groups (no disease vs. prostate disease) were analyzed using a Mann-Whitney U test. The differences between the three groups (no disease, BPH, PCa) were analyzed using a Kruskal-Wallis test. The correlation between PSA and CCL11 were analyzed using a Pearson correlation test. The p-values of less than 0.05 were set to be statistically significant. Receiver operator characteristic (ROC) analyses were performed to examine the sensitivity and specificity of CCL11 as a predictor of prostate diseases and to calculate the areas under the curves (AUCs) and confidence intervals. The data are presented as the means±SEMs, unless otherwise specified.

RESULTS

The study population consisted of 228 subjects; 45

Table 1. Patient demographic, clinical and CCL11 data

	No disease	BPH	PCa	Disease (BPH or PCa)
Total number	45	139	44	183
Mean age (y)	39.1±1.26	64.0±0.07	73.3±0.18	66.2±0.06
PSA (ng/ml)	0.9±0.00	2.6±0.03	90.2±5.75	23.9±0.71
CCL11 (pg/ml)	57.6±8.24	72.9±3.15	80.0±4.91	74.6±3.64
TRUS (ml)	-	39.3±0.13	52.2±0.98	41.9±0.14

All the data are expressed as means±standard errors except for total numbers. PSA differed significantly between the three group (Kruskal-Wallis test, $p<0.0001$). Forty-four with prostate cancer included 8 patients with Gleason scores (GS) <6, 17 patients with GS of 7 and 19 patients with GS of 8 or more.
CCL11: CC-chemokine ligand 11, BPH: benign prostatic hyperplasia, PCa: prostate cancer, PSA: prostate-specific antigen, TRUS: transrectal ultrasonography.

controls without prostatic disease (PSA<1 ng/ml), 139 patients with BPH, and 44 with PCa, including 8 patients with Gleason scores (GS) <6, 17 patients with GS of 7, and 19 patients with GS of 8 or more (Table 1).

We enquired if serum CCL11 levels were different between prostatic disease (PCa and BPH) patients and individuals without prostatic disease. The serum CCL11 levels for men without prostatic disease averaged 57.6 pg/ml, whereas those for men with BPH and PCa averaged 72.9 and 80.0 pg/ml, respectively (Table 1, Fig. 1). The CCL11 levels were significantly higher in PCa patients (p=0.012) and in BPH patients (p=0.001) than in healthy participants (Fig. 1A); however, there was no significant difference between the BPH (72.9 pg/ml) and PCa (80.0 pg/ml) levels (Fig. 1A, B).

We also investigated if CCL11 is a predictor of PCa aggressiveness. Thus, we measured CCL11 in PCa patients with different GS, and found that the CCL11 levels in men with GS 6 and with GS 8 or higher were similar: 73.1 pg/ml and 76 pg/ml, respectively. The level measured in patients with GS 7 scores was 94.4 pg/ml, which was actually higher than that measured in patients with GS 6 and GS 8. Therefore, we conclude that CCL11 levels do not differ between individuals with low, intermediate, and high risks of PCa (Fig. 1C).

Moreover, we performed a ROC analysis of serum CCL11 levels to test whether CCL11 might be a marker for distinguishing between prostatic tumors (BPH, PCa) and disease-free prostates. The areas under the ROC curves were 0.661 (p=0.001) and 0.654 (p=0.012) for BPH and PCa, respectively, compared with normal men (Fig. 2). Additionally, AUC was 0.659 for prostate disease compared with normal men. This suggests that CCL11 can distinguish between prostatic tumorous conditions (BPH, PCa) and normal individuals without prostatic disease. Three AUC levels were ranged from 0.6 to 0.7, which indicate poor accuracy. Further study is required to distinguish the methods between normal and prostate disease.

We note that the PSA levels differed significantly between the three groups (normal, BPH & PCa), but there was no correlation between CCL11 and serum PSA levels (by Pearson correlation).

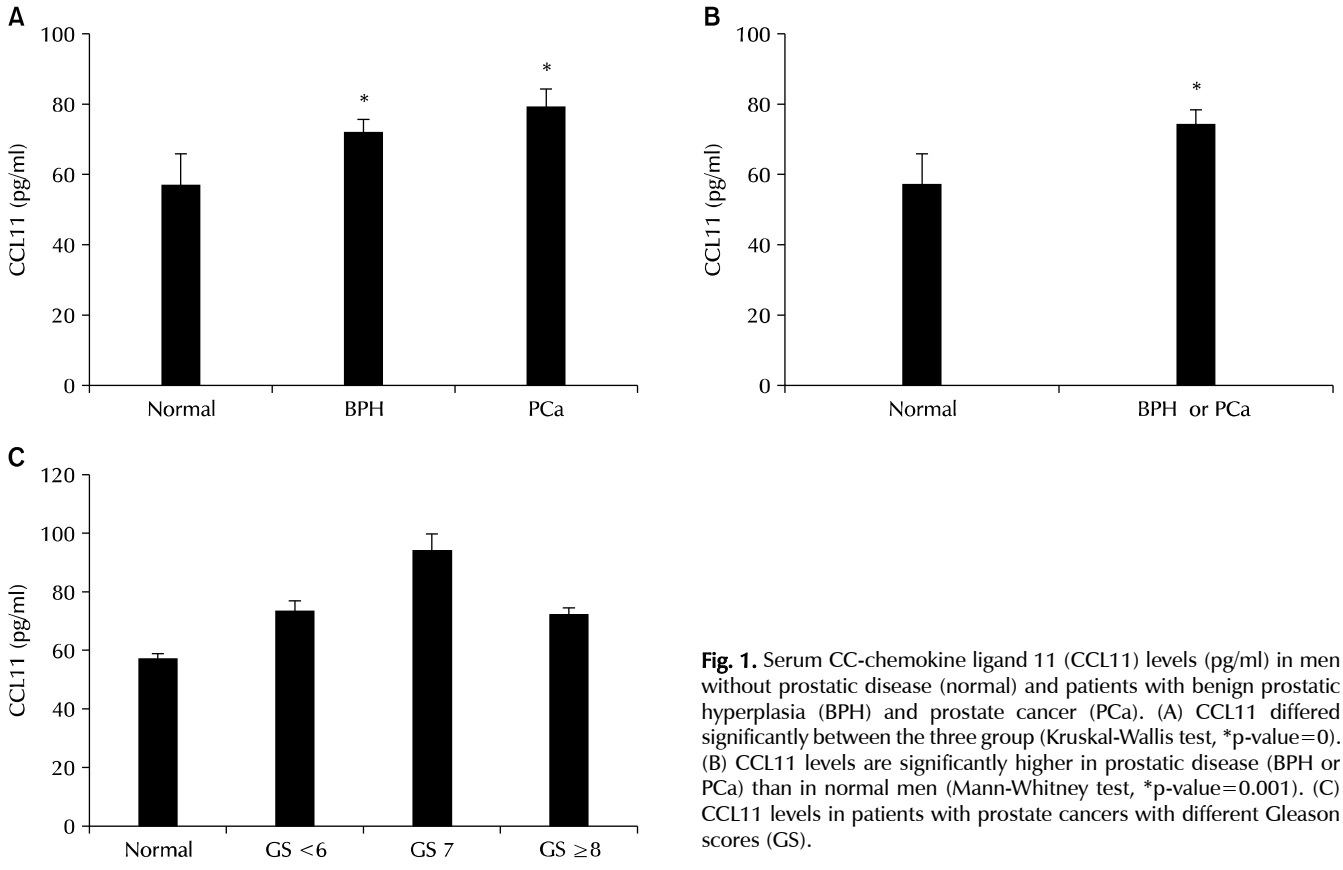

Fig. 1. Serum CC-chemokine ligand 11 (CCL11) levels (pg/ml) in men without prostatic disease (normal) and patients with benign prostatic hyperplasia (BPH) and prostate cancer (PCa). (A) CCL11 differed significantly between the three group (Kruskal-Wallis test, *p-value=0). (B) CCL11 levels are significantly higher in prostatic disease (BPH or PCa) than in normal men (Mann-Whitney test, *p-value=0.001). (C) CCL11 levels in patients with prostate cancers with different Gleason scores (GS).

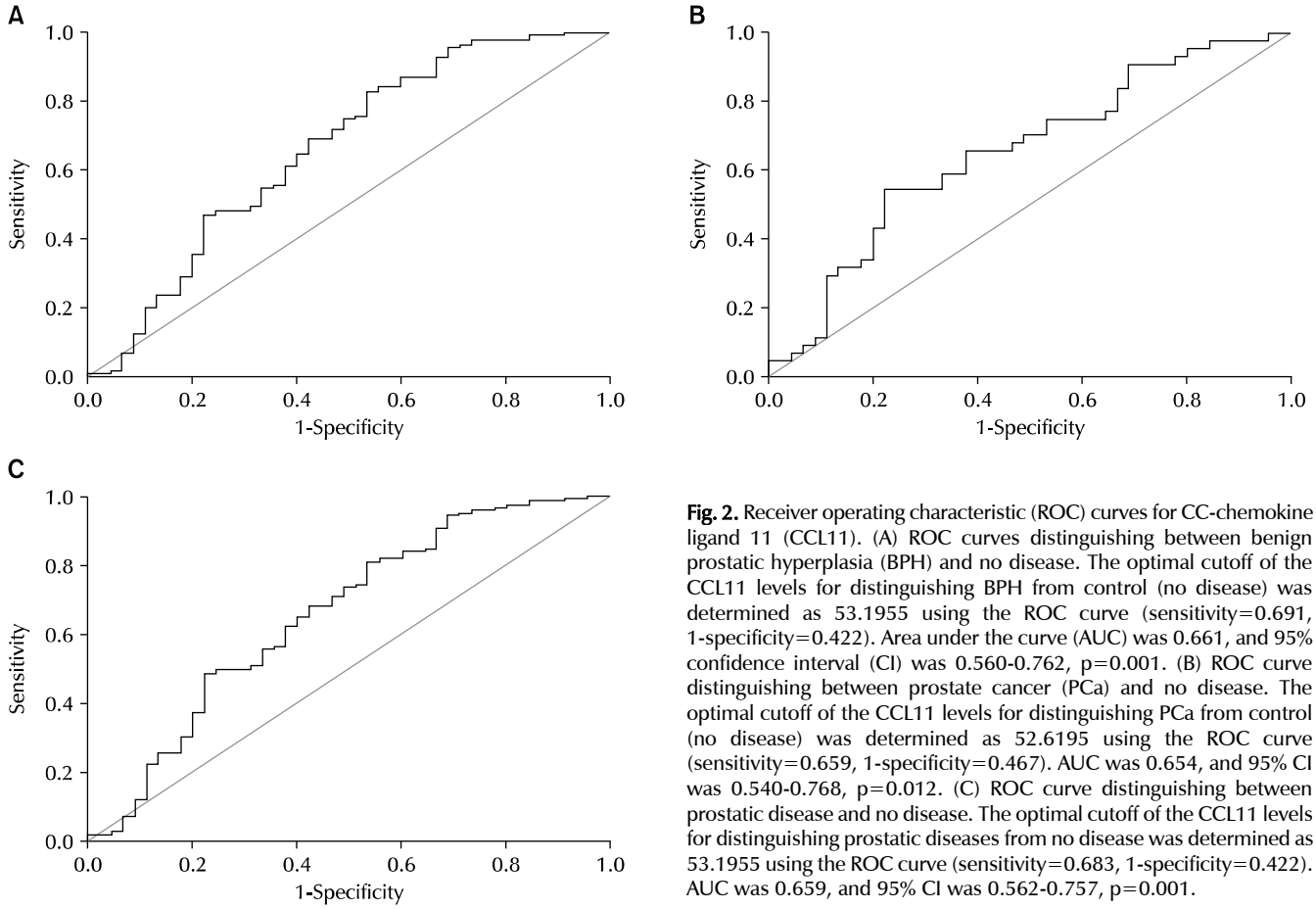

Fig. 2. Receiver operating characteristic (ROC) curves for CC-chemokine ligand 11 (CCL11). (A) ROC curves distinguishing between benign prostatic hyperplasia (BPH) and no disease. The optimal cutoff of the CCL11 levels for distinguishing BPH from control (no disease) was determined as 53.1955 using the ROC curve (sensitivity=0.691, 1-specificity=0.422). Area under the curve (AUC) was 0.661, and 95% confidence interval (CI) was 0.560-0.762, p=0.001. (B) ROC curve distinguishing between prostate cancer (PCa) and no disease. The optimal cutoff of the CCL11 levels for distinguishing PCa from control (no disease) was determined as 52.6195 using the ROC curve (sensitivity=0.659, 1-specificity=0.467). AUC was 0.654, and 95% CI was 0.540-0.768, p=0.012. (C) ROC curve distinguishing between prostatic disease and no disease. The optimal cutoff of the CCL11 levels for distinguishing prostatic diseases from no disease was determined as 53.1955 using the ROC curve (sensitivity=0.683, 1-specificity=0.422). AUC was 0.659, and 95% CI was 0.562-0.757, p=0.001.

DISCUSSION

BPH, a condition involving the abnormal progressive proliferation of prostatic stromal tissue and the cause lower urinary tract syndrome, is common in elderly men [5,6]. PCa is the second leading cause of cancer deaths in many western countries and accounted for more than 28,000 deaths in the United States in 2013 [7]. However, key questions remain about how best to diagnose this cancer.

Numerous pro-inflammatory cytokines play a major role in inflammatory disease and carcinogenesis.

Inflammation of the prostate, including activation of inflammatory cells and production of cytokines, appears to be an important causal factor in BPH and PCa [8,9]. Thus, various cytokines such as interleukin (IL)-8, IL-1, IL-6, tumor necrosis factor-α, CXCL5, CXCL12 have been tried as biomarkers for prostatic diseases [10-12].

Very little is known regarding the potential role(s) of CCL11 in prostate pathology. CCL11 is known as a chemotactic factor that binds to the CCR3 receptor, activates the mitogen-activated protein kinase pathway, and stimulates cellular invasion by regulating the expression of matrix metalloproteinase-3 [13]. CCL11 has been proposed by two research groups to be a serum marker for PCa; Agarwal et al. [3] suggested that serum CCL11 may be a useful diagnostic tool to help distinguish between prostatic enlargement and PCa among men demonstrating low, but detectable, serum PSA value; Heidegger et al. [4] reported that CCL11 may serve as a diagnostic marker to distinguish between disease-free prostate condition and PCa.

However, most PCa are first found during screening with a PSA blood test, alone or in combination with a digital rectal exam, followed by a diagnostic biopsy and potentially imaging if there is a suspicion of cancer spread. When PCa is diagnosed, PSA levels are used in tumor staging and for tracking cancer progression [14]. PSA screening is associated with a significant decline in prostate cancer-specific mortality in the US over the past two decades [15,16], although the recommendation by the United States Preventive Services Task Force 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 transcripts and protein markers as diagnostic markers for PCa [3].

Several markers, including prostate-specific membrane antigen, hepsin, a-methylacyl-coenzyme A racemase, telomerase, serine protease TMPRSS2, d-catenin, and prostate-specific non-coding RNA called prostate cancer gene 3 (PCA3, formerly known as DD3), have been identified and tested to date; these markers, when used alone or in combination with serum PSA, have been determined to be variously useful as diagnostic or prognostic markers of PCa [17-20].

CCL11 is a ligand for the G-protein coupled receptor, CCR3, which is expressed on eosinophils, basophils, and Th2 helper T-cells [21-23]. In addition to its role as a chemo-attractant, CCL11 has been implicated in different eosinophilic inflammatory diseases, such as atopic dermatitis, allergic rhinitis, sinusitis, asthma, ulcerative colitis, and in several other gastrointestinal disorders, as well as in parasitic infections [24-28]. Moreover, the levels of serum or plasma CCL11 as diagnostic or prognostic cancer markers have been described in high-grade renal cell carcinomas, high-risk neuroblastoma, as well as in head and neck small cell carcinomas [29-31].

In this study, BPH and PCa showed a significantly increased CCL11 concentration than normal prostate. In addition, CCL11 of PCa is higher than that of BPH, although there is not a significant difference between the two group. The CCL11 levels do not differ between individuals with low, intermediate, and high risks of PCa classified by the GS.

However, the CCL11 plasma level was reported to increase with age in humans [32,33]. A limitation of our study is that the age of normal men (control group) without prostatic diseases is younger than that of prostatic diseases. The reason for this is that it is difficult to get a serum from prostatic diseases-free men of old age. CCL11 of the normal group showed lower levels than that of prostatic diseases, and the lower levels of CCL11 in normal men would be partially explained by young age. Further study is required to compare the CCL11 levels between prostatic tumor and control men of similar age to get a better understanding of using CCL11 as a biomarker for PCa. Taken together, this result suggested that CCL11 may be a supplementary diagnostic tool to differentiate between normal prostate and prostate tumor.

CONCLUSIONS

CCL11 may be helpful in distinguishing between normal disease-free prostate and prostatic diseases, such as BPH and PCa.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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