

## Original Article

# Association of serum EPCA-2 level with prostate cancer in Chinese Han population

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Received June 27, 2015; Accepted July 29, 2015; Epub August 1, 2015; Published August 15, 2015

**Abstract:** Background: Early prostate cancer antigen 2 (EPCA-2), a kind of nuclear matrix protein, may relate to prostate cancer. However, the association of EPCA-2 level in serum with prostate diseases has not been clarified in Chinese Han population. Methods: EPCA-2 and prostate-specific antigen (PSA) levels in serum were detected by enzyme linked immunosorbent assay in 116 patients with prostate cancer (PCa), 342 patients with benign prostatic hyperplasia (BPH), and 174 healthy controls (Control) in Chinese population. Associations of serum EPCA-2 and PSA level with prostate diseases were analyzed by ANOVA. Comparison of diagnostic effect for prostate cancer between EPCA-2 and PSA was evaluated by Receiver Operator Curve, Chi-square test, and others. Results: Serum EPCA-2 and PSA levels in PCa group were significantly higher than BPH and Control group (EPCA-2:  $F=200.05$ ,  $P<0.01$ ; PSA:  $F=210.65$ ,  $P<0.01$ ). However, EPCA-2 levels in the prostate cancers with different pathological grade were no significant difference. Furthermore, for detection of prostate cancer, EPCA-2 had a sensitivity of 81.9% and a specificity of 87.6%. Conclusions: Serum EPCA-2 could be used as a potential serological marker to diagnose prostate cancer in Chinese Han population, which was more specific than PSA and did not associate with pathological grades of prostate cancer.

**Keywords:** EPCA-2, PSA, prostate cancer, serum marker, Chinese

## Introduction

Prostate cancer has become one of the most common nonskin malignancies among men with an incidence of approximately 0.01% worldwide, whose mortality has also dramatically increased in China [1]. Early diagnosis is beneficial to improve the therapeutic effect and reduce the mortality of prostate cancer. At present digital rectal examination and serum prostate specific antigen (PSA) level assay are the most important techniques to diagnose prostate cancer initially. However, the confirmed diagnosis relies on the pathobiology resulted from aspiration biopsy or surgical operation of prostate.

The total PSA, introduced in the late 1980s, is now the most widely used test to screen prostate cancer [2]. However, its level may also be seen increased in some benign diseases such as benign prostate hyperplasia and prostatitis [3]. Komatsu et al [4] reported that PSA could

also elevate in physiological fluctuation. Therefore, it is a controversial topic that the prostate cancer diagnosis or prediction only uses the elevation of serum PSA level [5]. A more sensitive and specific biological marker for prostate cancer diagnosis is in need of exploration.

Nuclear matrix protein is a main component of nuclear matrix, which is the insoluble structural framework inside cell nucleus to determine nuclear shape and higher order DNA organization [6]. While the function and structure changes of nuclear matrix protein have been found in the carcinogenesis of cells [7]. Alan et al have shown the characteristic differences of nuclear matrix protein patterns among a series of prostate tumors and the specificity in rat sex-accessory tissues [8].

Early prostate cancer antigen (EPCA) is one of the nuclear matrix protein associated with prostate cancer. Dhir et al [9] had identified that

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EPCA expressed only in the prostate of patients with prostate cancer, not in others without the disease. Furthermore, previous studies had shown that EPCA could accurately diagnose for prostate cancer even at the earlier stage of carcinogenesis for prostate cancer [9, 10]. Two EPCA subtypes have been identified, the first one also designated as EPCA, and the second as EPCA-2 [10]. The serum EPCA level was firstly analyzed by Paul et al [7] in a series of 46 individuals, and found significantly higher in prostate cancer patients than other populations. Furthermore, the study also revealed that the serum EPCA, at a cutoff of 1.7, had a sensitivity of 92% for prostate cancer patients, a specificity of 100% for healthy controls [7].

However, the association of serum EPCA-2 level with prostate diseases, and the diagnosis values of EPCA-2 for prostate cancer were unclarified. In the present study, we identified the association of serum EPCA-2 level with prostate diseases and the differentiation of prostate cancers in Chinese samples. Furthermore, we also examined the potential uses of EPCA-2 in blood samples for the clinical management of prostate cancer.

### Materials and methods

#### Subjects

One hundred and sixteen patients with localized prostate cancer (PCa), three hundred and forty-two patients with benign prostatic hyperplasia (BPH, diagnosis referred the AUA guideline [11]) were recruited from Xinxiang Central Hospital and Chinese PLA General Hospital during October 2011 to September 2014. The patients with prostate cancer were further stratified by Gleason Grading System [12] (2-4 scores as well differentiated prostate cancer, WDPCa,  $N=34$ ; 5-7 scores as moderately differentiated prostate cancer, MDPCa,  $N=42$ ; 8-10 scores as poorly differentiated prostate cancer, PDPCa,  $N=40$ ). All pathological diagnosis and Gleason score were evaluated independently by at least two pathologists and clinicians in Chinese PLA General Hospital or Xinxiang Central Hospital.

One hundred and seventy-four healthy controls (Control) without prostate diseases history and complex disorders including hypertension, immunological mediated disease, diabetes, and tumors were selected from Beijing and Henan Province. All participators in the study

were Chinese Han. The mean age of the PCa group was  $71.2\pm 6.3$  (WDPCa:  $71.4\pm 6.5$ ; MDPCa:  $71.4\pm 6.5$ ; PDPCa:  $71.0\pm 6.2$ ), BHP was  $71.2\pm 4.9$ , and Control was  $71.0\pm 5.5$ . No significant difference was existed in the age among each group.

All subjects consented to participate in the study after reviewing the informed consent. The study was approved by both the Institutional Review Board of the Xinxiang Central Hospital and Chinese PLA General Hospital.

#### Blood collection and preparation

Venous blood was collected from patients and healthy controls which had not been treated by catheterization, digital rectal examination, transrectal ultrasonography, cystoscopy, and prostate massage in a week, prostate biopsy in a month. Peripheral blood samples (5 mL for each) from each subject were drawn into vacutainer tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) between 6:00 and 7:00 AM to avoid circadian fluctuation of the measured parameters [13]. Serum was obtained through centrifugation at 3000 rpm for 20 min, and was then used to evaluate the indirect enzyme linked immunosorbent assay (ELISA) for EPCA-2 and PSA, or stored at  $-70^{\circ}\text{C}$  until required.

#### Assessment of serum EPCA-2 and PSA levels

Serum levels of EPCA-2 and PSA were evaluated using a Human EPCA-2 ELISA kit (CK-E11846, RapidBio, Inc., USA) and Human PSA ELISA kit (KB11690, Jianglai, Ltd., PRC). Microtiter strips coated with a monoclonal antibody against EPCA-2 and PSA were used. Samples, including 50  $\mu\text{l}$  standards of known EPCA-2 or PSA concentrations, or 50  $\mu\text{l}$  EPCA-2 or PSA antigens with diluent were added to the wells. The strips were incubated for 30 min at room temperature, followed by washed in 200  $\mu\text{l}$  of wash buffer for 3 times. A horseradish peroxidase (HRP) conjugated monoclonal antibody specific for EPCA-2 or PSA was then added and the strips was incubated for 1 h at room temperature. Subsequently, the strips were washed to remove all unbound enzyme, and 50  $\mu\text{l}$  of color reagents A and B mixed together for incubation within 15 min. The intensity of the color was measured at 450 nm by Microplate Reader (PHoMo, Autobio, Ltd., PRC), that directly indicated the concentration of EPCA-2 or PSA presented in the samples.

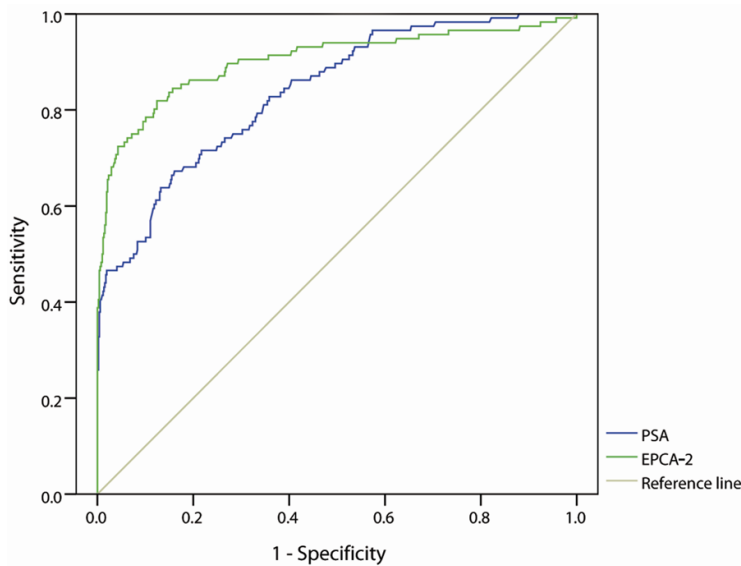
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**Table 1.** Association analysis of serum EPCA and PSA levels with prostatic diseases (Mean  $\pm$  SD)

Group	N	Age	EPCA-2 (ng/mL)	PSA (ng/mL)
PCa	116	71.2 $\pm$ 6.3	41.60 $\pm$ 18.59	22.78 $\pm$ 23.33
BPH	342	71.2 $\pm$ 4.9	16.42 $\pm$ 9.34	5.54 $\pm$ 4.70
Control	174	71.0 $\pm$ 5.5	12.42 $\pm$ 7.28	1.96 $\pm$ 2.41
F		0.58	200.05	210.65
P		0.75	<0.01	<0.01

**Table 2.** Association analysis of serum EPCA and PSA levels with differentiations of prostate cancer (Mean  $\pm$  SD)

Differentiation	N	Age	EPCA-2 (ng/mL)	PSA (ng/mL)
Well	34	71.4 $\pm$ 6.5	43.54 $\pm$ 18.11	17.06 $\pm$ 20.99
Moderate	42	71.4 $\pm$ 6.3	40.93 $\pm$ 19.22	20.42 $\pm$ 19.84
Poor	40	71.0 $\pm$ 6.2	40.67 $\pm$ 18.67	30.12 $\pm$ 27.00
F		0.11	2.01	5.44
P		0.94	0.37	0.07



**Figure 1.** Receiver operator characteristic (ROC) curves for EPCA-2 and PSA in 632 samples. The area under ROC curve of EPCA-2 and PSA is 0.903 (95% CI: 0.877-0.925) and 0.838 (95% CI: 0.807-0.866), respectively.

### Data analysis

Statistical analyses were performed using the SPSS 18.0 statistical package. The correlations among age and the serum levels of EPCA-2 and PSA were assessed by Spearman's rho correlation coefficient [14]. The differences of serum EPCA-2 and PSA levels among PCa, BPH, and Control, even among WDPCa, MDPCa, and

PDPCa were compared using analysis of variance (ANOVA). The diagnosing accuracies of EPCA-2 and PSA were summarized using receiver operating characteristic curves, sensitivity, specificity, positive/negative likelihood ratio, positive/negative predicted value, and coincidence rate [15, 16]. Furthermore, the comparison of diagnosis effects between EPCA and PSA for prostate cancer was performed with Chi-square test [17]. Data including age and serum EPCA-2 and PSA levels were expressed as mean  $\pm$  SD. All of the criterions for significance was set at  $P < 0.05$ .

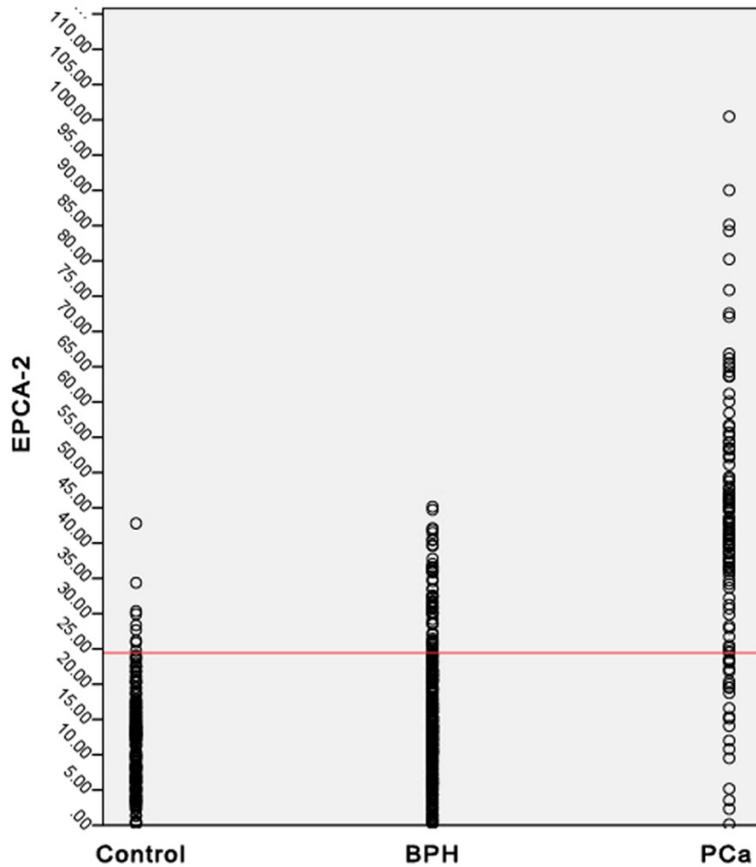
### Results

To evaluate the correlations of age with serum EPCA-2 and PSA levels, Spearman rho correlation coefficient was used and revealed that age was not significantly related to EPCA-2 and PSA ( $r = 0.025$  and  $0.008$ ,  $P = 0.537$  and  $0.843$ , respectively). But the correlation analysis also suggested that serum EPCA-2 level was moderately related to PSA ( $r = 0.320$ ,  $P < 0.01$ ).

The serum EPCA-2 and PSA levels were higher in patients with prostate cancer ( $41.60 \pm 18.59$  and  $22.78 \pm 23.33$  ng/mL) than patients with benign prostatic hyperplasia ( $16.42 \pm 9.34$  and  $5.54 \pm 4.70$  ng/mL) and healthy controls ( $12.42 \pm 7.28$  and  $1.96 \pm 2.41$  ng/mL), and the difference was significant ( $F = 200.05$  and  $210.65$ , respectively, both  $P < 0.01$ , **Table 1**). A total of 116

patients with prostate cancer including 34 well, 42 moderate, and 40 poor differentiation of pathology enrolled in the present study. However, none of the serum EPCA-2 and PSA levels was significant difference among the patients with well ( $43.54 \pm 18.11$  and  $17.06 \pm 20.99$  ng/mL), moderate ( $40.93 \pm 19.22$  and  $20.42 \pm 19.84$  ng/mL), and poor ( $40.67 \pm 18.67$  and  $30.12 \pm 27.00$  ng/mL) differentiated pros-

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**Figure 2.** Distribution of serum EPCA-2 levels in 632 samples. The samples consist of 174 healthy control (first column), 342 patients with benign prostatic hyperplasia (second column), and 116 patients with prostate cancer (third column) are presented. Red line represents the cutoff value of 24.44 ng/mL for EPCA-2.

**Table 3.** Comparison of the positive rate for prostate cancer detection between EPCA-2 and PSA (Cut off: 2.5 ng/mL)

Chi-square test	PCa			Non-PCa		
	PSA +	PSA -	Total	PSA +	PSA -	Total
EPCA-2 +	85	10	95	48	16	64
EPCA-2 -	21	0	21	227	225	452
Total	106	10	116	275	241	516

tate cancer ( $F=2.01$  and  $5.44$ ,  $P=0.37$  and  $0.07$ , respectively, **Table 2**).

The receiver operating characteristic curves revealed a remarkable rise of the area under the curve (AUC) for EPCA-2 (0.903, 95% CI: 0.877-0.925) compared with PSA (0.838, 95% CI: 0.807-0.866) as shown in **Figure 1**. The most outlying point closest to the top left corner (the maximum Youden) was selected to be the optimal cutoff value. Then 24.44 ng/mL was determined as the cutoff value for detect-

ing prostate cancer of EPCA-2 established with these pilot sets of Chinese Han population. In addition, the cutoff value of PSA in the present study was set at 2.5 or 4.0 ng/mL followed the National Comprehensive Cancer Network (NCCN) Practice Guidelines for Prostate Cancer (v.1.2001). The serum EPCA-2 level distributions of 632 samples were shown in **Figure 2**, and the red line represented the cut off value.

After establishing the cut off value of EPCA-2, 95 of 116 patients with prostate cancer and 64 of 516 samples without prostate cancer were evaluated to be serum EPCA-2 positive samples. However, 106 or 100 of 116 patients with prostate cancer and 275 or 215 of 516 samples without prostate cancer were respectively evaluated to be serum PSA positive sample when the cutoff value of PSA set at 2.5 or 4.0 ng/mL (**Tables 3 and 4**). Therefore, serum EPCA-2 level at a cut-off of 24.44 ng/mL had a sensitivity of 81.9% and specificity of 87.6% compared with a sensitivity of 91.4% or 86.2 and specificity of 46.7% or 58.3% for PSA with cut off value of 2.5 or 4.0 ng/mL (**Table 5**). Chi-square tests indicated that the specificity of EPCA-2 for detecting prostate cancer was significantly

higher than PSA (Cutoff value of 2.5 ng/mL: chi-square value =181.5,  $P<0.01$ ; 4.0 ng/mL: chi-square value =107.7,  $P<0.01$ ; **Tables 3-5**). But the sensitivities of EPCA-2 and PSA were no significant difference (Cut off value of 2.5 ng/mL: chi-square value =3.226,  $P=0.07$ ; 4.0 ng/mL: chi-square value =0.485,  $P=0.49$ ; **Tables 3-5**). In addition, the positive/negative likelihood ratio, positive/negative predicted value, and coincidence rate of EPCA-2 and PSA were showed in **Table 5**.

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**Table 4.** Comparison of the positive rate for prostate cancer detection between EPCA-2 and PSA (Cut off: 4.0 ng/mL)

Chi-square test	PCa			Non-PCa		
	PSA +	PSA -	Total	PSA +	PSA -	Total
EPCA-2 +	81	14	95	35	29	64
EPCA-2 -	19	2	21	180	272	452
Total	100	16	116	215	301	516

**Table 5.** Comparison of the diagnostic indicators between EPCA-2 and PSA for prostate cancer detection

Diagnostic Indicators	PSA (2.5 ng/mL)	PSA (4.0 ng/mL)	EPCA-2 (24.44 ng/mL)
Sensitivity (%)	91.4	86.2	81.9 <sup>###</sup>
Specificity (%)	46.7	58.3	87.6 <sup>*,**</sup>
Positive Likelihood Ratio	1.71	2.08	6.60
Negative Likelihood Ratio	0.18	0.24	0.21
Positive Predicted Value (%)	27.8	31.8	59.8
Negative Predicted Value (%)	96.0	95.0	95.6
Coincidence Rate (%)	42.7	46.4	69.1

#: Comparison between EPCA-2 and PSA (2.5 ng/mL), preponderant chi-square value =3.226,  $P=0.07$ ; ##: Comparison between EPCA-2 and PSA (4.0 ng/mL), preponderant chi-square value =0.485,  $P=0.49$ ; \*: Comparison between EPCA-2 and PSA (2.5 ng/mL), preponderant chi-square value =181.5,  $P<0.01$ ; \*\*: Comparison between EPCA-2 and PSA (4.0 ng/mL), preponderant chi-square value =107.7,  $P<0.01$ .

### Discussion

In the present study, we focused on EPCA-2, a potential serum tumor marker in prostate cancer, and systematically investigated its concentration in 632 serum samples from 116 patients with prostate cancer, 342 patients with benign prostatic hyperplasia, and 174 healthy controls. We first determined that the serum EPCA-2 and PSA levels were not affected by age. Furthermore, we observed that the serum EPCA-2 and PSA levels were associated with type of prostatic diseases. EPCA-2 had a higher specificity for detecting prostate cancer than PSA. All of these findings are entirely novel understanding for EPCA-2 in Chinese Han population.

Prostate specific antigen, a biomarker of prostate cancer, has been widely applied in clinical diagnosis and efficacy evaluation for prostate cancer [18]. However, the positive rate hovered around approximately 20% due to the increased PSA levels resulted by inflammation or benign hyperplasia of prostate [19, 20]. Furthermore, PSA could be detected expression in blood only

at the time that the barrier of prostate epithelial cell-vascular had been invaded and destroyed by carcinomas [18, 21]. To improve the positive rate of detection prostate cancer, several novel predicting biomarkers such as beta1,4-GalNAc-disialyl-Lc4 antigen (RM2) [22], prostate specific antigen density (PSAD) [23], and prostate specific antigen velocity (PSAV) [24] were introduced, but most of these predictors are also based on PSA.

The present study had revealed that the specificity of PSA for detecting prostate cancer was only approximately 50%, while EPCA-2 was 87.6% that may be more accurately for cancer detection. The EPCA-2 antigen was determined to structurally and functionally belong to the nuclear matrix proteins, which plays an essential role of regulators in DNA replication, transcription and gene expression [25]. Numerous studies, including both animal experiments and clinical studies, have demonstrated that the nuclear matrix proteins are specifically related to carcinogenesis of various organizations especially of prostate [26, 27]. Furthermore, another kind of nuclear matrix protein, EPCA, has been verified to be a potential predictor for detecting prostate cancer due to its high sensitivity and specificity [8, 28]. Taking together, we infer that EPCA-2 may be at least a potential biomarker for prostate cancer screening in Chinese Han samples. However, the associations of EPCA with EPCA-2, and the advantages of EPCA-2 compared with EPCA needs further explorations.

In summary, we presented the association of EPCA-2 with prostatic diseases, and revealed the potential values of EPCA-2 for prostate cancer detection in Chinese Han population, but limitations also existed. Firstly, the results of the present study were not in accordance with Leman's due to the different ethnic samples selection, ELISA kits, and other laboratorial conditions which would obviously affect the experimental results. Secondly, we only ana-

lyzed the parameter of diagnosis for prostate cancer, not including the treatment effects and prognosis, and further studies should be done to analyze more variables. Thirdly, because of the limitations from quality of cases and time-consuming, our sample size was inadequate that could easily led to false positive or negative results; hence other multi-center clinical studies were needed. Finally, whether EPCA-2 would be considered as a biomarker to determine the risk and prognosis of prostate cancer needs further explorations.

#### Acknowledgements

This study was funded by the Technology Development Program of Science & Technology Department of Henan (No. 122102310013).

#### Disclosure of conflict of interest

None.

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