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The Usefulness of Serum Human Kallikrein 11 for Discriminating between Prostate Cancer and Benign Prostatic Hyperplasia

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ABSTRACT

Prostate-specific antigen (PSA) is the most useful tumor marker for diagnosis and monitoring of prostate cancer (CaP). Recently, we developed a specific immunoassay for human kallikrein 11 (hK11), one of the kallikrein gene family members, and found that hK11 was highly expressed in prostatic tissue and could be detected in seminal plasma (E. P. Diamandis *et al.*, Cancer Res., *62:* 295–300, 2002). The aim of this study was to investigate whether serum hK11 levels could be used to discriminate CaP from benign prostatic hyperplasia (BPH). We analyzed for hK11, total PSA, and percentage of free PSA, 150 serum samples from men with histologically confirmed BPH ($n = 64$) or CaP ($n = 86$). Total and free PSA levels were measured by the Immulite PSA assay, and hK11 levels were measured by our previously published immunofluorometric assay. Serum hK11 levels and the hK11:total PSA ratio were both significantly lower in CaP patients than in BPH patients. In the subgroup of patients with percentage of free PSA less than 20, an additional 54% of BPH patients could have avoided biopsies by using the hK11:total PSA ratio. Receiver operating characteristic (ROC) curve analysis demonstrated that the hK11:total PSA ratio [area under the curve (*AUC*), 0.83] and percentage of free PSA (*AUC*, 0.83) were much stronger predictors of CaP than total PSA (*AUC*, 0.69). These preliminary data suggest that the hK11:total PSA ratio could be a useful tumor marker for CaP and could be combined with percentage of PSA to further reduce the number of unnecessary prostatic biopsies.

INTRODUCTION

CaP[2] is the most frequently diagnosed cancer in men in North America and its mortality rate is second only to lung cancer. Therefore, early diagnosis and monitoring of CaP is an important priority. PSA is widely used as the most reliable tumor marker established thus far (1, 2). However, nonmalignant prostatic diseases, especially BPH and acute prostatitis, also cause serum PSA elevation, thus complicating the diagnosis of CaP by PSA measurements alone. Analysis of the molecular forms of PSA improves specificity for CaP (3, 4). Especially, the determination of free PSA and its ratio to total PSA is now clinically established and is used to reduce the number of unnecessary prostate biopsies (5). Despite numerous efforts to further reduce unnecessary biopsies, false negative and false positive results still occur with high frequency.

The hK11 gene (*KLK11*) was originally isolated from human hippocampus as trypsin-like serine protease (TLSP; Ref. 6). We also found two alternative spliced variants of this gene, also known as *hippostasin* (7–9). With the official nomenclature, TLSP/hippostasin is now known as hK11. This protein is encoded by the *KLK11* gene, which belongs to the human kallikrein family along with PSA (hK3) and other kallikreins (10). We have previously demonstrated that hK11 protein is highly expressed in the prostate (8, 11). We have also developed an hK11-specific immunoassay and quantified hK11 in seminal plasma and prostatic tissue extracts. Our preliminary data showed elevated serum levels of hK11 in some advanced CaP patients (11). These findings led us to investigate the potential role of hK11 as an additional prostate tumor marker that may have the potential to enhance the specificity of current PSA testing.

In this study, we measured serum hK11 in a group of patients with BPH or CaP and whose total PSA and percentage free PSA values were also known. We then examined the usefulness of these variables, as well as their various combinations, in discriminating between these two clinical conditions.

MATERIALS AND METHODS

Study Population. Included in this study were serum samples from 150 male patients, 64 with BPH (median age 65) and 86 with CaP (median age 62), all histologically confirmed by biopsy. All of the patients were from the Department of Urology at University Hospital Charité, Humboldt University, Berlin, Germany. Total and percentage free PSA, measured by the Immulite method (Diagnostic Products Crop., San Diego, CA) were available for all of the samples. All of the sera were stored at −80°C until use.

The samples were collected with informed consent and the study was approved by the Institutional Review Board of the University Hospital Charité, Humboldt University.

Production of Monoclonal and Polyclonal Antibodies Against hK11. Purified recombinant hK11 protein was used to immunize rabbits and mice, to raise polyclonal and monoclonal antibodies as described elsewhere (11). hK11 was produced by expression in Pichia Pastoris (Invitrogen) and purified by SP Sepharose cation exchange column and reverse phase liquid chromatography.

Development of Immunofluorometric Assay for hK11. In this study, we used the hK11 assay described previously, with minor modifications (11). In brief, sheep antimouse IgG (Jackson Immunoresearch), diluted in coating buffer [containing 50 mM Tris (pH 7.8)], was dispensed into a 96-well white polystyrene microtiter plate (500 ng/100 μl/well) and was incubated overnight at room temperature. The plates were then washed three times with washing buffer that contained 9 g/liter NaCl and 0.5 g/liter Tween 20 in 10 mM Tris (pH 7.8). One hundred μl of anti-hK11 monoclonal antibody (100 ng) diluted in 6% BSA were added to each well and incubated with orbital shaking for 2 h at room temperature. The plates were washed six times with wash buffer. Fifty μl of hK11 calibrators (recombinant hK11 in 6% BSA) or samples were applied into each well along with 50 μl of 6% BSA. The plates were incubated for 2 h on an orbital shaker. After this step, we followed the procedure described previously (11). All of the samples were analyzed in triplicate. The detection limit of this assay is 0.1 μg/liter; for statistical analysis, all of the values below this limit were assigned a value of 0.05 μg/liter.

Statistical Analysis. The analysis of differences between measured or calculated parameters in the two groups, were performed with the nonparametric Mann-Whitney *U* test. Relationships between different variables were assessed by Spearman correlation coefficient. ROC curves were constructed for total PSA, percentage free PSA, and hK11:total PSA ratio, by plotting

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[2] The abbreviations used are: CaP, prostate cancer; BPH, benign prostatic hyperplasia; hK2, human glandular kallikrein; PSA, prostate-specific antigen; hK11, human kallikrein 11; CI, confidence interval; ROC, receiver operating characteristic; AUC, area under the (ROC) curve.

Table 1 *Descriptive statistics of various variables in serum of 64 BPH patients*

Variables	Mean ± SE	Range	Percentiles				
			10	25	50 (median)	75	90
hK11 (μg/liter)	0.41 ± 0.051	0.05–2.33	0.05	0.11	0.24	0.63	0.95
Total PSA (μg/liter)	5.61 ± 0.63	0.17–26.2	1.05	1.63	4.17	8.02	12.95
Free PSA (μg/liter)	1.22 ± 0.26	0.04–15.0	0.22	0.39	0.64	1.41	2.01
Percentage free PSA	22.3 ± 0.1.56	0.85–64.0	10.0	13.4	18.5	28.2	38.4
hK11:total PSA	0.14 ± 0.03	0.005–1.28	0.017	0.031	0.066	0.13	0.39

Table 2 *Descriptive statistics of various variables in serum of 86 CaP patients*

Variables	Mean ± SE	Range	Percentiles				
			10	25	50 (median)	75	90
hK11 (μg/liter)	0.16 ± 0.017	0.05–0.72	0.05	0.05	0.05	0.26	0.38
Total PSA (μg/liter)	9.34 ± 0.82	0.35–48.0	2.86	4.79	7.23	11.50	18.72
Free PSA (μg/liter)	1.03 ± 0.26	0.10–23.0	0.27	0.42	0.61	0.97	1.55
Percentage free PSA	10.9 ± 0.8	1.9–47.9	3.9	6.6	8.6	13.3	21.7
hK11:total PSA	0.030 ± 0.0053	0.002–0.31	0.004	0.006	0.013	0.036	0.062

sensitivity *versus* (1 − specificity), and the *AUC* were analyzed by the Hanley and McNeil method.

RESULTS

hK11 levels were measured in 150 serum samples from 86 patients with CaP and 64 patients with benign prostate hyperplasia with known total and percentage free PSA values. Descriptive statistics are summarized in Tables 1 and 2.

Total PSA values ranged from 0.17 to 26.2 μg/liter in BPH patients, with a mean ± SE of 5.61 ± 0.63 μg/liter and from 0.35 to 48.0 μg/liter in CaP patients, with a mean ± SE of 9.34 ± 0.82 μg/liter. Percentage free PSA levels ranged from 0.85 to 64.0 (mean ± SE, 22.3 ± 0.16) and from 1.9 to 47.9 (mean ± SE, 10.9 ± 0.8) in patients with BPH and CaP, respectively. hK11 concentrations ranged from 0.05 to 2.33 μg/liter (mean ± SE of 0.41 ± 0.051 μg/liter) in BPH patients and from 0.05 to 0.72 μg/liter (mean ± SE of 0.16 ± 0.017 μg/liter) in CaP patients. The distributions of hK11 between the two groups were significantly different ($P < 0.001$). The mean value ± SE of hK11:total PSA ratio was 0.14 ± 0.03 (range, 0.005–1.28) in patients with BPH and 0.030 ± 0.0053 (range, 0.002–0.31) in patients with CaP. The distribution of hK11:total PSA ratio was significantly lower in CaP patients than in BPH patients ($P < 0.001$). At 90% sensitivity (hK11:total PSA ratio of 0.06) the specificity is 46.5% (Fig. 1). Furthermore, we analyzed the hK11:total PSA ratio in the subgroup of patients with percentage free PSA <20 (these patients would have undergone biopsy based on percentage free PSA). At a cutoff point of 0.05 (90% sensitivity), specificity was 51.5%. Eighteen of these 35 patients could have avoided biopsy based on this criterion (Fig. 2).

We found a weak positive correlation between hK11 and total PSA levels in the group of BPH patients (Spearman correlation coefficient, $r_s = 0.30$; $P = 0.015$). However, in CaP patients, a significant correlation between hK11 and other variables was not observed (data not shown).

We further investigated the discriminatory value of the hK11:total PSA ratio in relation to the total PSA and percentage free PSA values, by ROC curve analysis. We found that the hK11:total PSA ratio, overall, had about the same discriminatory potential as percentage free PSA (AUC = 0.83; 95% CI, 0.76–0.89 for the hK11:total PSA ratio and AUC = 0.83 with 95% CI, 0.76–0.89, for the percentage free PSA; Fig. 3). Both parameters were superior to total PSA (AUC = 0.69). ROC analysis was also performed in subgroups of patients stratified according to total PSA values. Among BPH and CaP patients with serum total PSA <4 μg/liter, the AUCs were 0.70 (95% CI, 0.35–0.87) and 0.75 (95% CI, 0.59–0.91) for hK11 and the hK11:total PSA ratio, respectively. hK11 was also statistically significant in discriminating between CaP and BPH in patients with total PSA of 4–10 μg/liter ("gray zone"; AUC = 0.74; 95% CI, 0.61–0.86). The hK11:total PSA ratio (AUC = 0.74; 95% CI, 0.62–0.86) was also found to be a significant predictor of cancer in patients within the gray zone. Among patients who had serum total PSA >10 μg/liter, the AUCs were 0.89 (95% CI, 0.37–1.00) and 0.90 (95% CI, 0.57–1.00) for hk11 and the hk11:total PSA ratio, respectively. Patients who had serum total PSA between 2 and 10 μg/liter (34 patients with BPH, 52 with CaP), the AUCs were 0.74 (95% CI, 0.63–0.85) for percentage free PSA (*%fPSA*) and 0.77 (95% CI, 0.67–0.87) for the hk11:free PSA ratio (Fig. 3*B*).

Univariate logistic regression models were developed to evaluate the value of total PSA, percentage free PSA, and hK11:total PSA

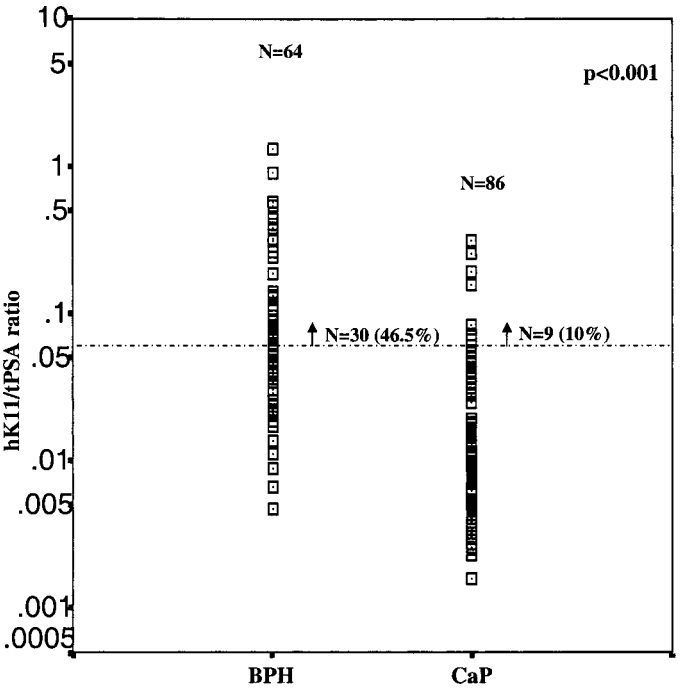

Fig. 1. Distribution of hK11:total PSA ratio in BPH and CaP patients. At 90% sensitivity (hK11:total PSA ratio of 0.06), the specificity is 48.5%. *P* was determined by the Mann-Whitney *U* test.

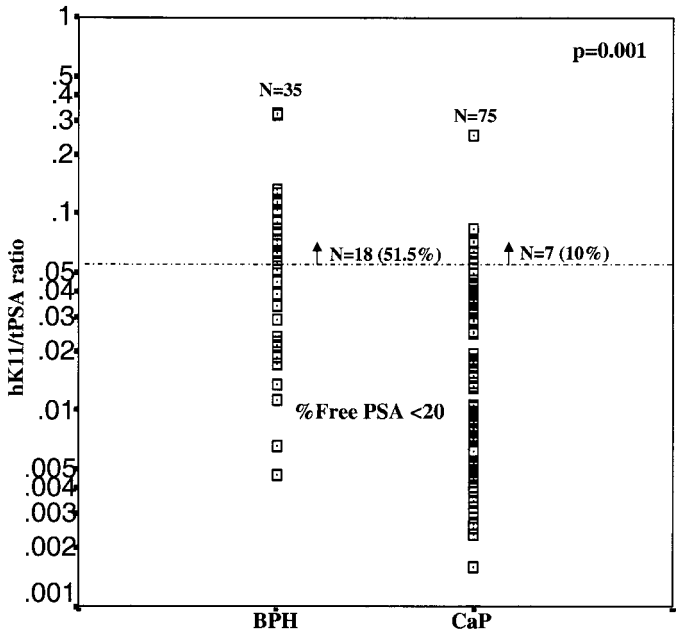

Fig. 2. Distribution of hK11:total PSA ratio in the subgroup of patients with percentage free PSA <20. At 90% sensitivity (hK11:total PSA ratio of 0.05), the specificity is 54%. *P* was determined by the Mann-Whitney *U* test.

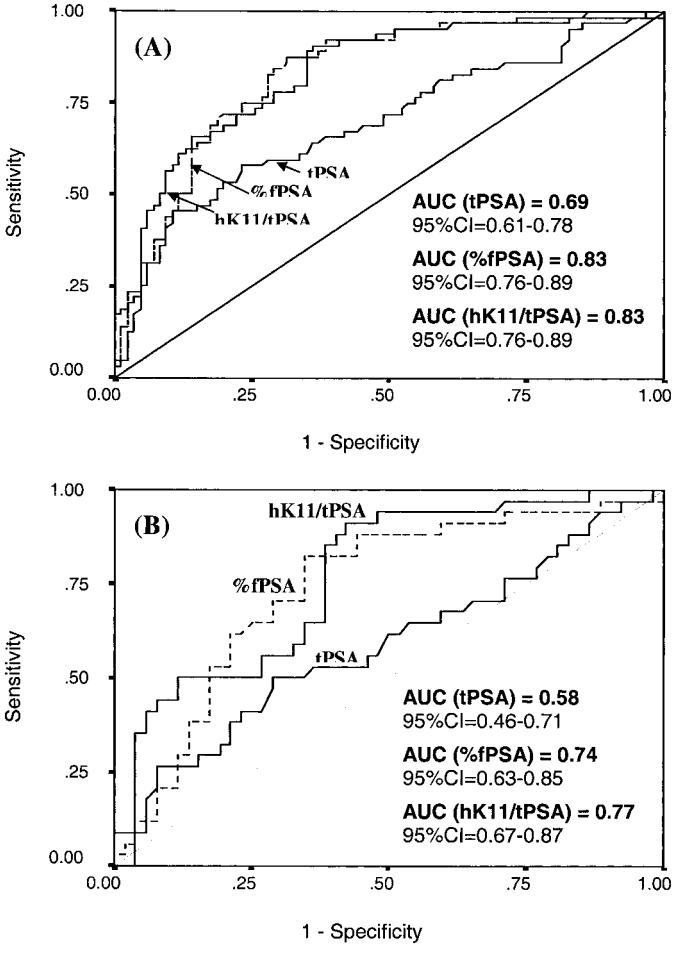

Fig. 3. ROC curves for total PSA (*tPSA*), percentage free PSA (*%fPSA*), and hK11:total PSA ratio, demonstrating the relative potential of each variable in the discrimination of BPH from CaP (*A*, the whole patient population; *B*, patients with total PSA values between 2 and 10 μg/liter).

ratios for discriminating between BPH and CaP (Table 3). These models demonstrated that patients with low levels of hK11:total PSA ratio were at increased risk to have CaP. In the multivariate analysis, the logistic regression models were adjusted for total PSA, percentage free PSA, and hK11:total PSA ratios. The percentage free PSA and hK11:total PSA ratios proved to be independent factors for discriminating between BPH and CaP patients (crude odds ratios, 0.90 and 0.91, and $P = 0.001$ and 0.031, respectively).

DISCUSSION

PSA is widely used as a tumor marker for CaP, but efficient discrimination between BPH and CaP with this test is not possible because of the lack of specificity. It has been reported that PSA molecular forms, including free PSA, can contribute to better discrimination (4, 12–14). hK2 is also useful for the differential diagnosis for CaP in the PSA range of 2.5–10 μg/liter (4, 15).

Recently, we developed an immunoassay for hK11 and found relatively large amounts of hK11 in seminal plasma and prostatic tissues (11). Thus, we speculated that hK11, like PSA and hK2, could also contribute to the discrimination between BPH and CaP.

In this study, we demonstrate for the first time that hK11 levels in serum are significantly lower in CaP patients than in BPH patients. The hK11:total PSA ratio is also significantly lower in CaP than in BPH. With the hK11:total PSA ratio and at 90% sensitivity, it could be possible to avoid ~50% of biopsies that could not have been avoided by the percentage-free-PSA test (percentage free PSA <20). These results demonstrate for the first time, that the combination of

Table 3 *Logistic regression analysis of BPH and CaP patients for predicting the presence of CaP*

	Univariate analysis		Multivariate analysis	
Covariate	Crude odds ratio	P^a	Crude odds ratio	P^a
Total PSA	1.12	0.002	1.06	0.089
Percentage free PSA	0.88	<0.001	0.90	0.001
hK11:total PSA	0.85	<0.001	0.91	0.031

[a] Test for trend.

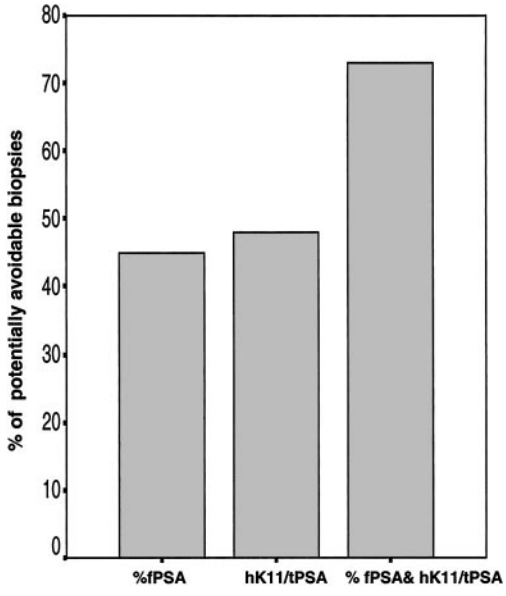

Fig. 4. Percentage of potentially avoidable biopsies by various biochemical parameters. Seventy-three % of BPH patients would have avoided biopsies by the combining of percentage free PSA (*%fPSA*) and the hK11:total PSA (*hK11/tPSA*) ratio (at 90% sensitivity).

percentage free PSA and the hK11:total PSA ratio could contribute to a better discrimination between BPH and CaP patients. Of 64 patients with BPH, and at 90% sensitivity, 29 patients (45%) would have avoided biopsy by percentage-free-PSA testing (>20% free PSA). From the remaining 35 patients, another 18 could have avoided biopsy by using the hK11:total PSA ratio (>0.05). When the two tests are combined, 47 BPH patients (73%) could have avoided biopsy at about 90% sensitivity (Fig. 4).

ROC curve analysis has demonstrated that the hK11:total PSA ratio has about the same discriminatory value as percentage free PSA, suggesting that hK11 could be an additional marker for CaP. However, these data should be considered preliminary because of the relatively small number of patients and the inability of the current hK11 immunoassay to quantify hK11 in the serum of ~25% of patients with BPH and ~50% of patients with CaP. Furthermore, in future studies, it will be desirable to use specimens with more closely matched total PSA values between BPH and CaP patients, to better define the usefulness of this new parameter in clinical practice.

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