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# Serum Human Glandular Kallikrein (hK2) and Insulin-Like Growth Factor I (IGF-I) Improve the Discrimination Between Prostate Cancer and Benign Prostatic Hyperplasia in Combination With Total and %Free PSA

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**BACKGROUND.** There is growing evidence describing an association of hK2 and IGFs with cancer. The aim of this study is to investigate the differences in serum levels of hK2 and IGFs in a large group of patients with benign prostatic hyperplasia (BPH) or prostatic carcinoma (CaP) and to examine the value of these variables, as well as their various combinations with PSA, for discriminating between these two clinical entities.
**METHODS.** Human glandular kallikrein 2 (hK2), insulin-like growth factor-1 (IGF-1), free and total PSA concentrations were measured with non-competitive immunological procedures. Receiver operating characteristic (ROC) analysis as well as univariate and multivariate logistic regression analysis were performed to investigate the potential utility of the various markers and their combinations for discriminating between BPH and CaP.
**RESULTS.** hK2 and IGF-1 concentrations were increased in CaP patients, in comparison to BPH patients. hK2/free PSA and free/total PSA ratios (area under the curve, AUC = 0.70) were stronger predictors of prostate cancer than the IGF-1/total PSA ratio (AUC = 0.56) in the group of patients with total PSA <4 μg/L. The hK2/free PSA ratio (AUC = 0.74) was found to have significant discriminatory value in patients with total PSA within the ''gray zone'' (4–10 μg/L). Multivariate logistic regression models confirmed the observed relationships and identified IGF-1/free PSA and hK2/free PSA as independent predictors of CaP.
**CONCLUSIONS.** hK2/free PSA and IGF-1/free PSA ratios may be useful adjuncts in improving patient selection for prostate biopsy. *Prostate 54: 220–229, 2003.*


*KEY WORDS:* **human glandular kallikrein 2 (hK2); insulin-like growth factor 1 (IGF-1); free PSA; total PSA; prostate cancer; benign prostatic hyperplasia; differential diagnosis**

Abbreviations used: BPH, benign prostatic hyperplasia; CaP, prostate cancer; hK2, human glandular kallikrein 2; IGF-1, insulin-like growth factor 1; PSA, prostate specific antigen; ACT, $\alpha_1$-antichymotrypsin; SE, standard error; CI, confidence interval.

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## INTRODUCTION

Prostate cancer (CaP) is the most frequently diagnosed cancer in men in the United States and its mortality rate is second only to lung cancer. Therefore, early diagnosis and monitoring of prostate cancer is an important priority. This clinical need is considerably enhanced by the measurement of serum prostate-specific antigen (PSA), the most reliable tumor marker established so far [1,2]. However, the analysis of total PSA levels in serum lacks sensitivity and specificity for optimal detection of early prostate cancer, especially in the 4 to 10 μg/L ''gray zone,'' where it cannot discriminate between patients with CaP and benign prostatic hyperplasia (BPH). Approximately 20–30% of prostate cancers are missed when a cut-off value of 4 μg/L is employed [3,4]. In addition, among patients with PSA values between 4 and 10 μg/L, only 25% will be found to have prostate cancer [5]. These significant limitations of PSA testing invariably result in a diagnostic dilemma, leading to either failure of early cancer detection, or to unnecessary surgical procedures or treatment. Although the ratio of free/total PSA levels in serum is significantly reduced in CaP, and its determination is now used to increase the diagnostic accuracy of PSA testing, there is still a great need to further improve our ability to discriminate between BPH and prostate cancer [2,3,6].

Prostate cancers detected through PSA screening are more likely to be localized to the prostate, than tumors detected by digital rectal examination [7–9]. Furthermore, it has been shown that measurement of the different molecular forms of serum PSA helps to discriminate between prostate cancer and BPH [10–13]. PSA is present in serum predominantly bound to proteinase inhibitors ($\alpha_1$-antichymotrypsin, ACT, and $\alpha_2$-macroglobulin), but there is also a fraction of free-PSA, an uncomplexed form which is enzymatically inactive. Recent studies indicate that the free/total PSA ratio in serum is lower in patients with prostate cancer than in patients with BPH. Also, indirect evidence suggests that serological screening for early prostate cancer may be an effective method of reducing mortality, although prospective clinical trials are now in progress to confirm this hypothesis [6,9,11,12,14].

Human glandular kallikrein 2 (hK2) belongs to the same family of serine proteases as PSA (human tissue kallikrein gene family) and displays a strong structural homology to PSA. Moreover, these two kallikreins share many biochemical properties and they are both primarily (but not exclusively) prostate-localized and they are androgen-regulated [15–18]. Findings that hK2 cleaves proPSA (244 residues) to generate enzymatically active PSA (237 residues) suggest a physiological role of hK2 in the regulation of PSA [19–21]. The hK2 protein is predominantly expressed in prostate epithelial cells and is present in serum, making it a potential marker for prostate cancer, either alone or in combination with PSA [2,4,21–23].

Insulin-like growth factors (IGF-1 and IGF-2) are mitogenic and anti-apoptotic agents produced primarily by the liver and locally, by a wide variety of tissues. IGFs circulate mostly complexed with IGF binding protein-3 (IGFBP-3), which, in association with the acid-labile subunit (ALS) forms an approximately 150 kDa ternary complex [24–26]. Disregulation and/or overexpression of the IGF system have been long implicated in the etiology of both benign and malignant proliferative disorders [25,27–30]. Malignant cells of various origins have been shown to express various components of the IGF system [30–33], and increased IGF levels, as seen in acromegaly, have been found in association with benign prostatic hyperplasia (BPH) and colon cancer [34]. High levels of circulating IGF-1 have been identified as risk factors for the development of prostate, breast, and lung cancers [35], while overexpression of both IGF-1 and IGF-2 has been linked to colorectal cancer [36]. In prostate, both benign and malignant cells have been found to express IGFs and their respective receptors [29,37].

In view of the growing evidence describing an association of increased serum concentrations of hK2 and IGFs with prostate cancer, we investigated the differences in serum levels of these molecules in a large group of patients with BPH or CaP. We further analyzed the value of these variables, as well as their various combinations, for discriminating between these two clinical entities.

## MATERIALS AND METHODS

### Patient Population and Samples

Enrolled in this study were 345 candidates for prostate biopsy, admitted to the Department of Urology of the University-Hospital of Padova during the period from January 1992 to December 1997. After biopsy patients were subdivided into two groups on the basis of the histologic diagnosis: (a) patients with benign prostatic hyperplasia (BPH) (174 males aged 49–82; median age 65) and (b) patients with prostate cancer (171 males aged 51–91; median age 68). Serum samples were obtained under standardized conditions from patients who had received no treatment for prostate disease at the time of phlebotomy. All specimens were residuals from routine testing and were stored frozen at −70°C until analyzed. Our study is in accordance with the Helsinki declaration and was approved by the Institutional Review Boards of the University-Hospital of Padova.

### Immunoassays

Concentrations of total and free PSA were determined using the Immulite 2000 automated immunoassay system (Diagnostic Products Corporation, San Diego, CA). IGF-1 was assayed by ACTIVE$^{R}$ enzyme-linked immunosorbent assay (ELISA) kits from Diagnostic Systems Laboratories, Inc., Webster, TX [38]. hK2 was analyzed with a time-resolved immunofluorometric assay as previously described [39] [40].

The hK2 assay had a detection limit of 0.006 μg/L and less than 0.2% cross-reactivity with PSA. The IGF-1 assay had a detection limit 0.01 μg/L. hK2 and IGF-1 concentrations were below the detection limit of the assays in 5.3 and 4.4% of the samples, respectively. Two percents of the samples had both hK2 and IGF1 concentrations below the detection limit of the assays. For statistical analysis, we assigned the values of 0.003μg /L for hK2 and 0.005 μg/L for IGF-1, for all samples with undetectable levels.

### Statistical Analysis

A number of functions between hK2, IGF-1, and PSA were calculated and descriptive statistics for these variables were performed for each group of patients. Due to the fact that the distributions of total PSA, free PSA, IGF-1, and hK2 concentrations in the BPH and CaP patients were not Gaussian, the analyses of differences between these parameters, in the two groups, were performed with the non-parametric Mann–Whitney U test. Relationships between different variables were assessed by Spearman correlation coefficient. The ability of the variables to predict presence of prostate cancer was studied using univariate and multivariate unconditional logistic regression analysis. Receiver operating characteristic (ROC) curves were constructed for free/total PSA, IGF-1/free PSA, and hK2/free PSA ratios, by plotting sensitivity versus (1-specificity) and the areas under the ROC curves (AUC) were analyzed by the Hanley and McNeil method. This analysis was also performed in three groups of patients stratified according to total serum PSA levels: (1) <4.0; (2) 4.0–10.0; and (3) >10.0 μg/L. For all analyses, a $P$ value of $<0.05$ was considered statistically significant.

## RESULTS

### Concentration of Measured Variables in Serum of BPH and CaP Patients

hK2, IGF-1, total and free PSA were measured in 345 serum samples from 171 patients with prostate cancer and 174 patients with benign prostatic hyperplasia. In Tables I and II, we present the descriptive statistics for these variables as well as for the free/total PSA, hK2/free PSA, and IGF-1/free PSA ratios.

Total PSA values ranged from 0.26 to 32.5 μg/L in BPH patients, with a mean ± SE of 6.00 ± 0.38 μg/L and from 0.28 to 393 μg/L in CaP patients, with the mean ± SE value being 10.9 ± 1.5 μg/L. Values were significantly higher in CaP than BPH ($P = 0.003$). Free PSA levels ranged from 0.05 to 14.8 μg/L (mean ± SE = 1.17 ± 0.10 μg/L) and from 0.04 to 14.3 μg/L (mean ± SE = 0.97 ± 0.24 μg/L) in patients with benign and malignant prostatic disease, respectively. The differences were statistically significant ($P = 0.006$). hK2 ranged from undetectable to 0.55 μg/L with the mean ± SE being 0.094 ± 0.0078 μg/L in samples from BPH patients. In CaP patients, hK2 concentrations ranged between undetectable and 7.84 μg/L, with a mean ± SE value of 0.23 ± 0.051 μg/L. The distribution of hK2 values was statistically different between the two groups of patients ($P < 0.001$). IGF-1 values ranged from zero to 500 μg/L in BPH patients, with a mean ± SE of 103 ± 7.3 μg/L and from zero to 530 μg/L in CaP patients, with the mean ± SE of 141 ± 8.1 μg/L.

**TABLE I. Descriptive Statistics of Variables in Serum of 174 Patients With Benign Prostate Hyperplasia**

| | | | Percentiles | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Mean ± SE[a] | Range | 5 | 25 | 50 | 75 | 95 |
| Total PSA (μg/L) | 6.00 ± 0.38 | 0.26–32.5 | 0.52 | 3.28 | 4.72 | 6.7 | 17.1 |
| Free PSA (μg/L) | 1.17 ± 0.10 | 0.05–14.8 | 0.53 | 0.54 | 0.90 | 1.40 | 3.00 |
| hK2 (μg/L) | 0.094 ± 0.0078 | 0.003[b]–0.55 | 0.003 | 0.016 | 0.058 | 0.14 | 0.31 |
| IGF-1 (μg/L) | 103 ± 7.3 | 0.005[b]–500 | 1.00 | 66 | 95 | 130 | 221 |
| hK2/free PSA | 0.11 ± 0.0077 | 0.000–0.55 | 0.002 | 0.019 | 0.074 | 0.17 | 0.27 |
| IGF-1/free PSA | 209 ± 43 | 0.001–1050 | 0.008 | 50 | 95 | 206 | 693 |
| Free/total PSA | 0.21 ± 0.0078 | 0.031–0.74 | 0.083 | 0.14 | 0.18 | 0.26 | 0.41 |

[a]Standard error.
[b]Half of the assay detection limit.

**TABLE II. Descriptive Statistics of Variables in Serum of 171 Patients With Prostate Cancer**

| Variable | Mean ± SE[a] | Range | Percentiles | | | | |
|---|---|---|---|---|---|---|---|
| | | | 5 | 25 | 50 | 75 | 95 |
| Total PSA (μg/L) | 10.94 ± 1.46 | 0.28–393 | 1.75 | 4.14 | 5.49 | 8.25 | 41.1 |
| Free PSA (μg/L) | 0.97 ± 0.24 | 0.04–14.30 | 0.19 | 0.42 | 0.69 | 0.95 | 2.88 |
| hK2 (μg/L) | 0.23 ± 0.051 | 0.003[b]–7.84 | 0.003 | 0.062 | 0.13 | 0.20 | 0.54 |
| IGF-1 (μg/L) | 142 ± 8.1 | 0.005[b]–530 | 50 | 91 | 126 | 175 | 367 |
| hK2/free PSA | 0.23 ± 0.023 | 0.000–4.35 | 0.0015 | 0.071 | 0.20 | 0.29 | 0.50 |
| IGF-1/free PSA | 237 ± 17 | 0.008–1025 | 36 | 114 | 191 | 324 | 680 |
| Free/total PSA | 0.13 ± 0.0065 | 0.02–0.46 | 0.041 | 0.073 | 0.12 | 0.16 | 0.28 |

[a]Standard error.
[b]Half of the assay detection limit.

Values were significantly higher in patients with CaP than BPH ($P = 0.003$).

In general, the concentration of free PSA was lower, while the hK2 and IGF-1 concentrations were increased in CaP patients, when compared with the respective values in BPH patients. A number of functions between PSA, hK2, and IGF-1 (ratios, logarithmic ratios, differences) were calculated and evaluated for their discriminatory potential between BPH and CaP (data not shown). We found that the hK2/free PSA and the IGF-1/free PSA ratio had the most promising discriminatory potential and were evaluated further. The mean ± SE value of hK2/free PSA and the IGF-1/free PSA ratio were 0.11 ± 0.0077 and 209 ± 43 in patients with BPH, while they were 0.23 ± 0.023 and 237 ± 17 in patients with CaP, respectively ($P < 0.001$). $P$ values were calculated by Mann–Whitney U test.

With Spearman correlation analysis, IGF-1 was not correlated significantly with hK2, total or free PSA levels. However, hK2 correlated positively with free and total PSA levels in BPH ($r_s = 0.35$, $P < 0.001$ and $r_s = 0.30$, $P < 0.001$ respectively) and CaP patients ($r_s = 0.53$, $P < 0.001$ and $r_s = 0.18$, $P = 0.028$, respectively).

### Receiver Operating Characteristic (ROC) Analysis

ROC analyses were performed to show the relative potential of hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios for discriminating prostate cancer and benign prostatic hyperplasia. Free/total PSA ratio (AUC, 0.73; 95% confidence intervals, CI, 0.66–0.79), hK2/free PSA (AUC, 0.68; 95% CI, 0.63–0.78) and IGF-1/free PSA ratio (AUC, 0.68; 95% CI, 0.61–0.75) were found to be significant in discriminating CaP and BPH, in the whole patient population (Fig. 1).

ROC analysis was also performed in subgroups of patients stratified according to total PSA values. Among patients who have serum total PSA <4 μg/L, the areas under the curves were 0.70 (95% CI, 0.54–0.85), 0.72 (95% CI, 0.52–0.81) and 0.56 (95% CI, 0.40–0.71) for hK2/free PSA, the free/total PSA ratio and the IGF-1/free PSA ratio, respectively (Fig. 2). The ratio of IGF-1/free PSA was statistically significant in discriminating between CaP and BPH in patients with total PSA 4–10 μg/L (''gray zone'') (AUC, 0.75; 95% CI, 0.67–0.83) or total PSA >10 μg/L (AUC, 0.87; 95% CI, 0.71–0.99) (Figs. 3 and 4). hK2/free PSA ratio (AUC, 0.74; 95% CI, 0.66–0.82) was also found to be a significant predictor of cancer in patients that belong to the ''gray zone.'' The discriminatory value of hK2/free PSA (AUC, 0.72; 95% CI, 0.59–0.86), IGF-1/free PSA (AUC, 0.87; 95% CI, 0.71–0.99) and free/total PSA ratio (AUC, 0.78; 95% CI, 0.67–0.90) was found to be significant in the subgroup of patients with total serum PSA >10 μg/L.

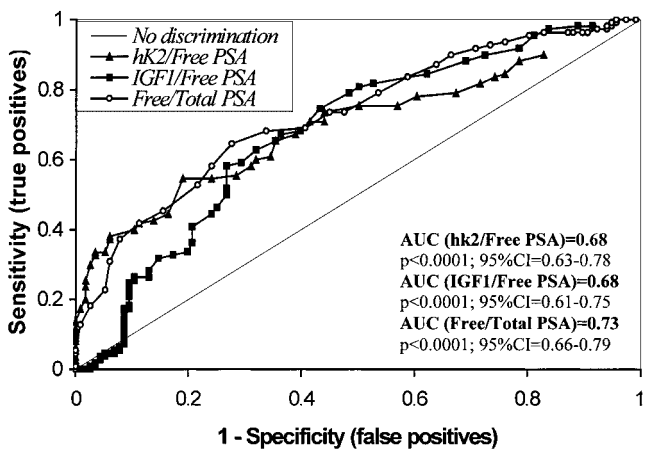


**Fig. 1.** Receiver operating characteristics (ROC) curves for hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios, demonstrating the relative potential of each variable in the discrimination of BPH from CaP in the whole patient population. CI, confidence interval.

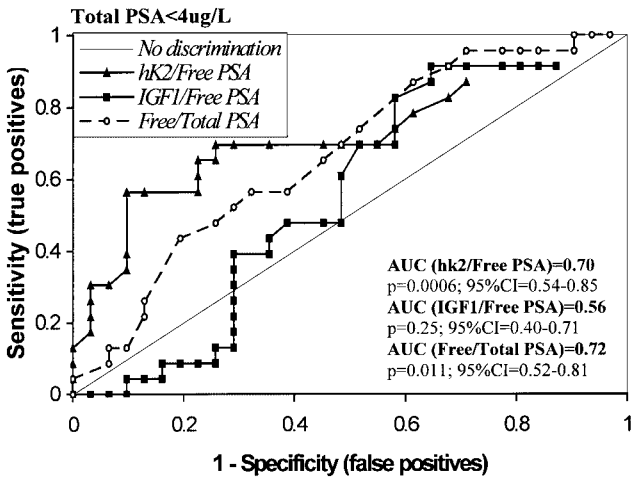


**Fig. 2.** ROC curves for hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios, demonstrating the relative potential of each variable in the discrimination of BPH from CaP when total PSA levels are <4 μg/L.

The comparative ability of hK2/free PSA, IGF-1/free PSA, and free/total PSA ratio in differentiating between BPH and CaP at selected cut-off points is summarized in Table III. With an IGF-1/free PSA cut-off value of 20 (sensitivity = 95% and specificity = 25%), 25% of biopsies could have been avoided in BPH patients, while 5% of the cancers would have been missed, in patients with total serum PSA >10 μg/L. Similarly, with an hK2/free PSA cut-off value of 0.23 (sensitivity = 30% and specificity = 95%), 30% of cancers might have been detected by biopsy, while 5% of BPH patients would have been subjected to an unnecessary biopsy, in patients with serum total PSA <4 μg/L.

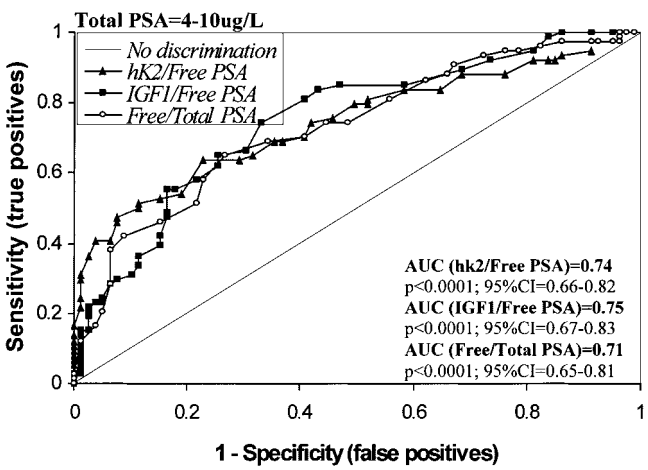


**Fig. 3.** ROC curves for hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios, demonstrating the relative potential of each variable in the discrimination of BPH from CaP when total PSA range from 4 – 10 μg/L.

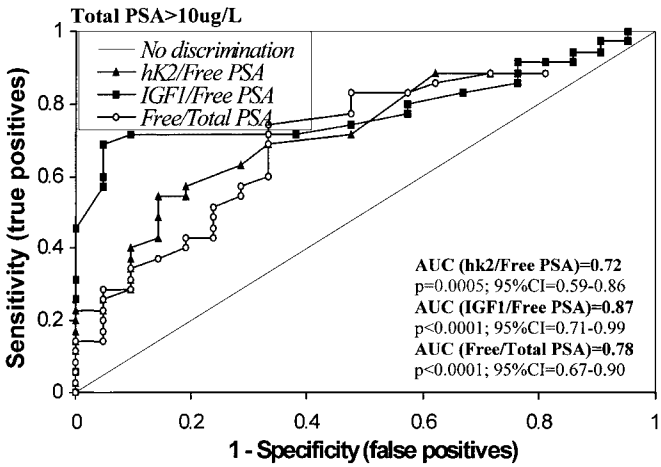


**Fig. 4.** ROC curves for hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios, demonstrating the relative potential of each variable in the discrimination of BPH from CaP when total PSA levels are >10 μg/L.

### Univariate and Multivariate Analysis

Univariate logistic regression models were developed to evaluate the value of hK2/free PSA, IGF-1/free PSA, and free/total PSA ratios for discriminating between BPH and CaP (Table IV). These models have demonstrated that patients with high levels of hK2/free PSA ratio and/or IGF-1/free PSA were at increased risk to have prostate cancer. In the multivariate analysis, the logistic regression models were adjusted for free/total PSA ratio, hK2/free PSA ratio and, IGF-1/free PSA. The free/total PSA ratio proved to be an independent factor for discriminating between BPH and CaP patients (crude odds ratio = 0.52, $P < 0.001$). hK2/free PSA ratio significantly added to the prognostic power of this multivariate model (crude odds ratio = 2.85, $P = 0.003$). hK2/free PSA was also found to be an independent variable in subgroups of patients with serum total PSA <4 μg/L or 4–10 μg/L (crude odds ratio = 1.31 and 7.69, $P = 0.037$ and <0.001, respectively). IGF-1/free PSA proved to be an independent factor in patients with total PSA > 10 μg/L (crude odds ratio = 8.42, $P = 0.025$) while it did not significantly add to the prognostic power of the multivariate models in subgroup of patients with total PSA 4–10 μg/L or <4 μg/L (Table IV).

To further investigate the discriminatory value of the hK2/free PSA ratio in relation to the IGF1/free PSA ratio, another logistic regression model was developed adjusted only for these two ratios. We calculated log likelihood scores for this multivariate logistic regression model, which incorporates both of the ratios, for each patient. For these data, the crude odds ratio and the 95% CI were found to be 2.72 and 1.48–3.91, respectively.

**TABLE III. Comparison of Sensitivity and Specificity at Selected Cut-Off Points**

| Parameter | Cut-off | Sensitivity (%) | Specificity (%) | CaP value |
|---|---|---|---|---|
| All patients (N = 345) | | | | |
| f/tPSA | 0.28 | 95 | 18 | <Cut-off |
| | 0.09 | 23 | 95 | |
| hK2/fPSA | 0.00 | 95 | 17.5 | >Cut-off |
| | 0.27 | 34 | 95 | |
| IGF-1/fPSA | 30 | 95 | 19 | >Cut-off |
| | 630 | 24 | 95 | |
| Total PSA <4 μg/L (N = 92) | | | | |
| f/tPSA | 0.27 | 95 | 29 | <Cut-Off |
| | 0.09 | 9 | 95 | |
| hK2/fPSA | 0.00 | 95 | 29 | >Cut-Off |
| | 0.23 | 30 | 95 | |
| IGF-1/fPSA | 0.00 | 95 | 13 | >Cut-Of |
| | 960 | 5 | 95 | |
| Total PSA 4–10 μg/L (N = 189) | | | | |
| f/tPSA | 0.26 | 95 | 24 | <Cut-Off |
| | 0.09 | 20 | 95 | |
| hK2/fPSA | 0.04 | 95 | 19 | >Cut-Off |
| | 0.26 | 41 | 95 | |
| IGF-1/fPSA | 40 | 95 | 23 | >Cut-Off |
| | 280 | 25 | 95 | |
| Total PSA >10 μg/L (N = 64) | | | | |
| f/tPSA | 0.36 | 95 | 10 | <Cut-Off |
| | 0.10 | 68 | 95 | |
| hK2/fPSA | 0.00 | 95 | 15 | >Cut-Off |
| | 0.18 | 26 | 95 | |
| IGF-1/fPSA | 20 | 95 | 25 | >Cut-Off |
| | 80 | 45 | 95 | |

fPSA, free PSA; tPSA, total PSA.

## DISCUSSION

In this study, we analyzed serum samples from 345 men with prostatic disease. Patients were stratified according to their total serum PSA concentrations into three groups: <4 μg/L, 4–10 μg/L, and >10 μg/L. Catalona et al. [6] reported that a 25% free PSA cut-off detected 95% of cancers while avoiding 20% of biopsies, in a cohort of patients with total PSA levels of 4–10 μg/L. Our data are quite similar. At the same sensitivity level (95%), the specificity is 24%, at a free/total PSA ratio of 26% (Table III).

IGFs are endocrine, paracrine and autocrine hormones that play significant roles in cellular growth and differentiation. Although accumulating evidence has implicated involvement of the IGF system in cellular carcinogenesis [31–33], the proposed association has not been invariably confirmed. Prostate carcinogenesis might be more closely dependent on disregulation of the IGF system [27,37]. Predictably, regulation of IGF's action depends on the integrated effects of the systemic/locally produced IGFs, various cell surface receptors and proteases that cleave the IGF binding proteins and, thus, modulate their bioactivities. The profound effects of diseased tissue-associated physiological changes and factors such as nutrition, genetics and aging on the rate of IGF production [28,41] further compound this complexity. As the systemic levels of IGFs could be influenced by multiple variables, serum determinations may not consistently reflect disease status if the masking effects of non-disease influences are not carefully considered. The latter may be exemplified by the reported non-significant association of IGF-1 with prostate and breast cancer risk in older individuals [42, 43].

Kallikreins are serine proteases with diverse physiological functions, which are expressed in various tissues [15–17,44–49]. Accumulating evidence indicates that members of the expanded kallikrein gene family are associated with various malignancies [50–58]. Early

**TABLE IV. Logistic Regression Analysis of BPH and CaP Patients for Predicting the Presence of Prostate Cancer**

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| Covariate | Crude odds ratio | *P*-value* | Crude odds ratio | *P*-value* |
| All patients (N = 345) | | | | |
| Free/total PSA ratio | 0.12 | <0.001 | 0.52 | <0.001 |
| hK2/free-PSA ratio | 9.11 | <0.001 | 2.85 | 0.003 |
| IGF-1/Free-PSA ratio | 1.89 | 0.025 | 1.44 | 0.068 |
| Total PSA <4 μg/L (N = 92) | | | | |
| Free/total PSA ratio | 0.33 | 0.002 | 0.30 | 0.012 |
| hK2/free-PSA ratio | 2.12 | 0.035 | 1.31 | 0.037 |
| IGF-1/free-PSA ratio | 1.52 | 0.045 | 1.44 | 0.84 |
| Total PSA 4–10 μg/L (N = 189) | | | | |
| Free/total PSA ratio | 0.13 | 0.009 | 0.21 | 0.005 |
| hK2/free-PSA ratio | 10.67 | 0.001 | 7.69 | <0.001 |
| IGF-1/free-PSA ratio | 9.76 | 0.006 | 4.02 | 0.28 |
| Total PSA >10 μg/L (N = 64) | | | | |
| Free/total PSA ratio | 0.65 | 0.21 | 0.55 | 0.16 |
| hK2/free-PSA ratio | 8.52 | 0.009 | 2.52 | 0.082 |
| IGF-1/free-PSA ratio | 17.5 | 0.001 | 8.42 | 0.025 |

*Test for trend.

studies demonstrated that human glandular kallikrein 2 (hK2) is present in serum of patients with elevated PSA, suggesting that it might be a new prostate cancer marker [4,22,59,60]. Darson et al [61] found that hK2 expression is more tumor-associated, while PSA is expressed at higher levels in the non-cancerous part of the prostate gland. Magklara et al. [62] later found that both prostate kallikreins, PSA and hK2, are expressed more in non-cancerous, in comparison to matched cancerous prostatic tissues. Studies are now underway to investigate the molecular form(s) of hK2 that will provide the most useful clinical information with respect to both screening and staging of prostate cancer. The concentration of the precursor form of hK2 (prohK2) was found to be raised in the serum of prostate cancer patients [39,61].

We found that the discriminatory value of hK2 and IGF-1 alone, between benign and malignant prostatic diseases was absent in patients with PSA values within the diagnostic ''gray zone'' and low in the two other patients groups. This is in agreement with previously published data [4,63–65]. Recent studies have reported that the ratio of hK2/free PSA is higher in CaP patients than in non-cancer subjects [66].

To examine the potential clinical utility of hK2/free PSA and IGF-1/free PSA ratios for discriminating between benign prostate hyperplasia and prostate cancer in patients with moderately elevated, low and clearly elevated PSA levels, we performed statistical analysis of these variables. The hK2/free PSA and IGF-1/free PSA ratio are significantly elevated in a proportion of prostate cancer patients, suggesting that these ratios may have potential as new prostatic markers. Of interest is the finding defined with a cut-off value of IGF-1/free PSA of 20 (sensitivity = 95% and specificity = 25%). Twenty five percents of biopsies might have been avoided in BPH patients, while 5% of the cancers would have been missed, in patients with total serum PSA >10 μg/L. Similarly, with an hK2/free PSA cut-off value of 0.23 (sensitivity = 30% and specificity = 95%), 30% of cancers might have been detected by biopsy, while 5% of BPH patients would have undergone an unnecessary biopsy, in patients with serum total PSA <4 μg/L (Table III). We also constructed ROC curves (Figs. 1 and 4) and performed multivariate analysis to further investigate the potential of the hK2/free PSA and IGF-1/free PSA ratios in the differentiation between the two diseases (Table IV). These variables seem promising. However, no calculated parameter was found to be better than free/total PSA ratio at sensitivity levels above 80%. Logistic regression analysis suggested that the combined use of the two calculated ratios may improve discrimination but not at high sensitivity. In the restricted concentration ranges the number of patients is small for this type of analysis. Larger studies are required further to elucidate the putative improved discrimination of the calculated parameters.

It is now recommended that all patients with total PSA >10 μg/L and all patients with total PSA >4 μg/L and %free PSA <25% be biopsied [2,6,13]. Thus, even if the hK2/free PSA and and/or IGF-1/free PSA ratios are elevated, in this group of patients, suggesting that they are very likely to have cancer, they will still be biopsied for confirmation. Consequently, the number of unnecessary biopsies will not be reduced if the ratios of hK2/free PSA and IGF-1/free PSA are used in patients with total PSA >4 μg/L. However, when total PSA is <4 μg/L, biopsy is not recommended [6]. In Tables III and IV, we present data supporting the notion that patients with increased hK2/free PSA and/or IGF-1/free PSA ratio and total PSA <4 μg/L are at a significantly increased risk of having prostate cancer. Although further studies are required to confirm the finding, it seems that these patients with total PSA between 2–4 μg/L who have hK2/free PSA ratio >0.23 may be good candidates for biopsy since they have a greater than 30% chance of having cancer.

## CONCLUSIONS

We investigated the potential utility of hK2 and IGF-1 in the discrimination between prostate cancer and benign prostate hyperplasia in the groups of patients with total PSA <4, 4–10, and >10 μg/L. We found that hK2 and IGF-1 may play a useful diagnostic role but only in combination with free PSA. This approach might identify a subgroup of patients with low total PSA (e.g., between 2–4 μg/L) who would probably have not been biopsied based on their total PSA alone, but are at high risk of having cancer and should be considered for biopsy. This modality is complementary to the free/total PSA ratio because the former has utility in ruling-in CaP, while the latter in ruling-out CaP. Prospective, population-based screening studies are required to further address these issues.

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## REFERENCES

1. Diamandis EP. Induction of prostate-specific antigen expression by synthetic progestins in patients with prostate and breast cancer [letter; comment]. Mayo Clin Proc 1998;73:706–707.
2. Scorilas A, Yu H, Soosaipillai AR, Gregorakis AK, Diamandis EP. Comparison of the percent free prostate-specific antigen levels in the serum of healthy men and in men with recurrent prostate cancer after radical prostatectomy. Clin Chim Acta 2000; 292:127–138.
3. Catalona WJ, Smith DS, Ornstein DK. Prostate cancer detection in men with serum PSA concentrations of 2.6 to 4.0 ng/mL and benign prostate examination. Enhancement of specificity with free PSA measurements [see comments]. Jama 1997;277:1452–1455.
4. Magklara A, Scorilas A, Catalona WJ, Diamandis EP. The combination of human glandular kallikrein and free prostate- specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA. Clin Chem 1999;45:1960–1966.
5. Catalona WJ, Smith DS, Ratliff TL, Basler JW. Detection of organ-confined prostate cancer is increased through prostate-specific antigen-based screening. Jama 1993;270:948–954.
6. Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, Richie JP, deKernion JB, Walsh PC, Scardino PT, Lange PH, Subong EN, Parson RE, Gasior GH, Loveland KG, Southwick PC. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: A prospective multicenter clinical trial Jama 1998;279:1542–1547.
7. Benson MC, Olsson CA. Prostate specific antigen and prostate specific antigen density. Roles in patient evaluation and management. Cancer 1994;74:1667–1673.
8. Carter HB, Pearson JD, Metter EJ, Brant LJ, Chan DW, Andres R, Fozard JL, Walsh PC. Longitudinal evaluation of prostate-specific antigen levels in men with and without prostate disease. JAMA 1992;267:2215–2220.
9. Catalona WJ, Smith DS, Wolfert RL, Wang TJ, Rittenhouse HG, Ratliff TL, Nadler RB. Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. JAMA 1995;274:1214–1220.
10. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha 1- antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. Cancer Res 1991;51:222–226.
11. Christensson A, Bjork T, Nilsson O, Dahlen U, Matikainen MT, Cockett AT, Abrahamsson PA, Lilja H. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. J Urol 1993;150:100–105.
12. Luderer AA, Chen YT, Soriano TF, Kramp WJ, Carlson G, Cuny C, Sharp T, Smith W, Petteway J, Brawer MK, et al. Measurement of the proportion of free to total prostate-specific antigen improves diagnostic performance of prostate-specific antigen in the diagnostic gray zone of total prostate-specific antigen. Urology 1995;46:187–194.
13. Stephan C, Jung K, Lein M, Sinha P, Schnorr D, Loening SA. Molecular forms of prostate-specific antigen and human kallikrein 2 as promising tools for early diagnosis of prostate cancer. Cancer Epidemiol Biomarkers Prev 2000;9:1133–1147.
14. Partin AW, Catalona WJ, Finlay JA, Darte C, Tindall DJ, Young CY, Klee GG, Chan DW, Rittenhouse HG, Wolfert RL, Woodrum DL. Use of human glandular kallikrein 2 for the detection of prostate cancer: Preliminary analysis. Urology 1999;54:839–845.
15. Magklara A, Scorilas A, Lopez-Otin C, Vizoso F, Ruibal A, Diamandis EP. Human glandular kallikrein in breast milk, amniotic fluid, and breast cyst fluid. Clin Chem 1999;45:1774–1780.
16. Obiezu CV, Giltay EJ, Magklara A, Scorilas A, Gooren L, Yu H, Diamandis EP. Dramatic suppression of plasma and urinary prostate specific antigen and human glandular kallikrein by antiandrogens in male-to-female transsexuals. J Urol 2000;163: 802–805.
17. Obiezu CV, Giltay EJ, Magklara A, Scorilas A, Gooren LJ, Yu H, Howarth DJ, Diamandis EP. Serum and urinary prostate-specific

antigen and urinary human glandular kallikrein concentrations are significantly increased after testosterone administration in female-to-male transsexuals. Clin Chem 2000;46:859–862.

18. Lovgren J, Rajakoski K, Karp M, Lundwall A, Lilja H. Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2. Biochem Biophys Res Commun 1997; 238:549–555.
19. Woodrum DL, French CM, Hill TM, Roman SJ, Slatore HL, Shaffer JL, York LG, Eure KL, Loveland KG, Gasior GH, Southwick PC, Shamel LB. Analytical performance of the Tandem-R free PSA immunoassay measuring free prostate-specific antigen. Clin Chem 1997;43:1203–1208.
20. Kumar A, Mikolajczyk SD, Goel AS, Millar LS, Saedi MS. Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2. Cancer Res 1997;57:3111–3114.
21. Takayama TK, Fujikawa K, Davie EW. Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein. J Biol Chem 1997;272:21582–21588.
22. Nam RK, Diamandis EP, Toi A, Trachtenberg J, Magklara A, Scorilas A, Papnastasiou PA, Jewett MA, Narod SA. Serum human glandular kallikrein-2 protease levels predict the presence of prostate cancer among men with elevated prostate-specific antigen. J Clin Oncol 2000;18:1036–1042.
23. Young CY, Andrews PE, Montgomery BT, Tindall DJ. Tissue-specific and hormonal regulation of human prostate-specific glandular kallikrein. Biochemistry 1992;31:818–824.
24. Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG. Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. J Clin Endocrinol Metab 1992;75:1046–1053.
25. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: Biological actions. Endocr Rev 1995;16:3–34.
26. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: Regulation and functions. Endocr Rev 1997;18:801–831.
27. Russell PJ, Bennett S, Stricker P. Growth factor involvement in progression of prostate cancer. Clin Chem 1998;44:705–723.
28. Rosen CJ, Pollak M. Circulating IGF-I: New perspectives for a new century. Trends Endocrinol Metab 1999;10:136–141.
29. Cohen P. Serum insulin-like growth factor-I levels and prostate cancer risk—Interpreting the evidence. J Natl Cancer Inst 1998;90:876–879.
30. Baserga R. The insulin-like growth factor I receptor: A key to tumor growth? Cancer Res 1995;55:249–252.
31. Rajah R, Valentinis B, Cohen P. Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53-and IGF-independent mechanism. J Biol Chem 1997;272:12181–12188.
32. Oh Y. IGF-independent regulation of breast cancer growth by IGF binding proteins. Breast Cancer Res Treat 1998;47:283–293.
33. Rajah R, Katz L, Nunn S, Solberg P, Beers T, Cohen P. Insulin-like growth factor binding protein (IGFBP) proteases: Functional regulators of cell growth. Prog Growth Factor Res 1995;6:273–284.
34. Orme SM, McNally RJ, Cartwright RA, Belchetz PE. Mortality and cancer incidence in acromegaly: A retrospective cohort study. United Kingdom Acromegaly Study Group. J Clin Endocrinol Metab 1998;83:2730–2734.
35. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M. Plasma insulin-like growth factor-I and prostate cancer risk: A prospective study. Science 1998;279:563–566.
36. Manousos O, Souglakos J, Bosetti C, Tzonou A, Chatzidakis V, Trichopoulos D, Adami HO, Mantzoros C. IGF-I and IGF-II in relation to colorectal cancer. Int J Cancer 1999;83:15–17.
37. Grimberg A, Cohen P. Growth hormone and prostate cancer: Guilty by association? J Endocrinol Invest 1999;22: 64–73.
38. Khosravi MJ, Diamandi A, Mistry J, Lee PD. Noncompetitive ELISA for human serum insulin-like growth factor-I. Clin Chem 1996;42:1147–1154.
39. Rittenhouse HG, Finlay JA, Mikolajczyk SD, Partin AW. Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): Two closely related, but distinct, kallikreins in the prostate. Crit Rev Clin Lab Sci 1998;35:275–368.
40. Black MH, Magklara A, Obiezu CV, Melegos DN, Diamandis EP. Development of an ultrasensitive immunoassay for human glandular kallikrein with no cross-reactivity from prostate-specific antigen. Clin Chem 1999;45:790–799.
41. Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J, Eriksson J, Toivanen L, Koskenvuo M, Leinonen P, Koistinen R, Seppala M. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 1996;98:2612–2615.
42. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M. Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 1998;351:1393–1396.
43. Wolk A, Mantzoros CS, Andersson SO, Bergstrom R, Signorello LB, Lagiou P, Adami HO, Trichopoulos D. Insulin-like growth factor 1 and prostate cancer risk: A population-based, case-control study. J Natl Cancer Inst 1998;90:911–915.
44. Yousef GM, Diamandis EP. The expanded human kallikrein gene family: Locus characterization and molecular cloning of a new member, KLK-L3 (KLK9). Genomics 2000;65:184–194.
45. Yousef G, Scorilas A, Magklara A, Soosaipillai A, Diamandis E.P. The PRSS6 gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family-Genomic characterization, mapping, tissue expression and hormonal regulation. Gene 2000;258:24–31.
46. Yousef GM, Chang A, Scorilas A, Diamandis EP. Genomic organization of the human kallikrein gene family on chromosome 19q13.3-q13.4. Biochem Biophys Res Commun 2000;276: 125–133.
47. Yousef GM, Scorilas A, Magklara A, Soosaipillai A, Diamandis EP. The KLK7 (PRSS6) gene, encoding for the stratum corneum chymotryptic enzyme is a new member of the human kallikrein gene family-genomic characterization, mapping, tissue expression and hormonal regulation. Gene 2000;254:119–128.
48. Yousef GM, Scorilas A, Diamandis EP. Genomic organization, mapping, tissue expression, and hormonal regulation of trypsin-like serine protease (TLSP PRSS20), a new member of the human kallikrein gene family. Genomics 2000;63:88–96.
49. Scorilas A, Diamandis EP, Levesque MA, Papanastasiou-Diamandi A, Khosravi MJ, Giai M, Ponzone R, Roagna R, Sismondi P, Lopez-Otin C. Immunoenzymatically determined pepsinogen C concentration in breast tumor cytosols: An

independent favorable prognostic factor in node- positive patients. Clin Cancer Res 1999;5:1778–1785.

50. Diamandis EP, Yousef GM, Luo I, Magklara I, Obiezu CV. The new human kallikrein gene family: Implications in carcinogenesis. Trends Endocrinol Metab 2000;11:54–60.
51. Yousef GM, Scorilas A, Jung K, Ashworth LK, Diamandis EP. Molecular cloning of the human kallikrein 15 gene (KLK15). Up-regulation in prostate cancer. J Biol Chem 2001;276:53–61.
52. Bharaj B, Scorilas A, Diamandis EP, Giai M, Levesque MA, Sutherland DJ, Hoffman BR. Breast cancer prognostic significance of a single nucleotide polymorphism in the proximal androgen response element of the prostate specific antigen gene promoter. Breast Cancer Res Treat 2000;61:111–119.
53. Bharaj B, Scorilas A, Giai M, Diamandis EP. TA repeat polymorphism of the 5alpha-reductase gene and breast cancer. Cancer Epidemiol Biomarkers Prev 2000;9:387–393.
54. Yousef GM, Kyriakopoulou LG, Scorilas A, Fracchioli S, Ghiringhello B, Zarghooni M, Chang A, Diamandis M, Giardina G, Hartwick WJ, Richiardi G, Massobrio M, Diamandis EP, Katsaros D. Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: A new Independent and favorable prognostic marker. Cancer Res 2001;61:7811–7818.
55. Obiezu CV, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Rigault de la Longrais IA, Arisio R, Diamandis EP. Higher human kallikrein gene 4 (KLK4) expression indicates poor prognosis of ovarian cancer patients. Clin Cancer Res 2001;7:2380–2386.
56. Luo LY, Katsaros D, Scorilas A, Fracchioli S, Piccinno R, Rigault de la, Longrais IA, Howarth DJ, Diamandis EP. Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. Clin Cancer Res 2001;7:2372–2379.
57. Magklara A, Scorilas A, Katsaros D, Massobrio M, Yousef GM, Fracchioli S, Danese S, Diamandis EP. The human KLK8 (neuropsin/ovasin) gene: Identification of two novel splice variants and its prognostic value in ovarian cancer. Clin Cancer Res 2001;7:806–811.
58. Kim H, Scorilas A, Katsaros D, Yousef GM, Massobrio M, Fracchioli S, Piccinno R, Gordini G, Diamandis EP. Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br J Cancer 2001;84:643–650.
59. Charlesworth MC, Young CY, Klee GG, Saedi MS, Mikolajczyk SD, Finlay JA, Tindall DJ. Detection of a prostate-specific protein, human glandular kallikrein (hK2), in sera of patients with elevated prostate-specific antigen levels. Urology 1997;49:487–493.
60. Darson MF, Pacelli A, Roche P, Rittenhouse HG, Wolfert RL, Young CY, Klee GG, Tindall DJ, Bostwick DG. Human glandular kallikrein 2 (hK2) expression in prostatic intraepithelial neoplasia and adenocarcinoma: A novel prostate cancer marker. Urology 1997;49:857–862.
61. Saedi MS, Hill TM, Kuus-Reichel K, Kumar A, Payne J, Mikolajczyk SD, Wolfert RL, Rittenhouse HG. The precursor form of the human kallikrein 2, a kallikrein homologous to prostate-specific antigen, is present in human sera and is increased in prostate cancer and benign prostatic hyperplasia. Clin Chem 1998;44:2115–2119.
62. Magklara A, Scorilas A, Stephan C, Kristiansen GO, Hauptmann S, Jung K, Diamandis EP. Decreased concentrations of prostate-specific antigen and human glandular kallikrein 2 in malignant versus nonmalignant prostatic tissue. Urology 2000;56:527–532.
63. Finne P, Auvinen A, Koistinen H, Zhang WM, Maattanen L, Rannikko S, Tammela T, Seppala M, Hakama M, Stenman UH. Insulin-like growth factor I is not a useful marker of prostate cancer in men with elevated levels of prostate-specific antigen. J Clin Endocrinol Metab 2000;85:2744–2747.
64. Becker C, Piironen T, Pettersson K, Bjork T, Wojno KJ, Oesterling JE, Lilja H. Discrimination of men with prostate cancer from those with benign disease by measurements of human glandular kallikrein 2 (HK2) in serum. J Urol 2000;163:311–316.
65. Khosravi J, Diamandi A, Mistry J, Scorilas A. Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 in benign prostatic hyperplasia and prostate cancer. J Clin Endocrinol Metab 2001;86:694–699.
66. Kwiatkowski MK, Recker F, Piironen T, Pettersson K, Otto T, Wernli M, Tscholl R. In prostatism patients the ratio of human glandular kallikrein to free PSA improves the discrimination between prostate cancer and benign hyperplasia within the diagnostic ''gray zone'' of total PSA 4 to 10 ng/mL. Urology 1998;52:360–365.