

Serum Human Glandular Kallikrein-2 Protease Levels Predict the Presence of Prostate Cancer Among Men With Elevated Prostate-Specific Antigen

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Purpose: We hypothesize that serum human glandular kallikrein-2 (hK2) levels predict the presence of prostate cancer among men prescreened by prostate-specific antigen (PSA).

Patients and Methods: We conducted a cross-sectional study of 324 men who had no history of prostate cancer and who were referred for prostate biopsy. PSA and hK2 levels were measured using specific nonisotopic immunometric techniques. Cases were patients who were diagnosed with adenocarcinoma of the prostate from biopsy, and controls were patients who had no evidence of cancer from biopsy. The odds ratio for detection of prostate cancer was determined for hK2 measurements, controlling for age, total-PSA level, digital rectal examination, and symptoms of urinary obstruction.

Results: Of 324 men, 159 (49.1%) had cancer. Mean hK2 levels and hK2:free-PSA ratios were significantly higher in cases than in controls (1.18 v 0.53 ng/mL, respectively, for hK2, $P = .0001$; 1.17 v 0.62 for hK2:free-PSA ratio, $P = .0001$). The crude odds ratio for

prostate cancer detection for patients in the highest quartile of hK2 level was 5.83 (95% confidence interval [CI], 2.8 to 12.1; $P = .0001$) compared with patients in the lowest quartile. The adjusted odds ratio was 6.72 (95% CI, 2.9 to 15.6; $P = .0001$). Similarly, the crude and adjusted odds ratios for prostate cancer detection using the hK2:free-PSA ratio were 7.36 (95% CI, 3.6 to 15.1; $P = .0001$) and 8.06 (95% CI, 3.7 to 17.4; $P = .0001$), respectively. These odds ratios were higher than that observed for prostate cancer detection by total-PSA level (2.73; $P = .03$).

Conclusion: Among men prescreened with PSA for prostate cancer, patients with high hK2 measurements have a five- to eight-fold increase in risk for prostate cancer, adjusting for PSA level and other established risk factors. hK2 measurements may be a useful adjunct to PSA in improving patient selection for prostate biopsy.

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PROSTATE CANCER is the most common malignancy in males and is the second leading cause of cancer death.^{1,2} Tumor stage is one of the most important prognostic factors for prostate cancer; patients with tumors confined to the prostate experience lower mortality rates than do patients with cancers that extend beyond the prostate gland.³⁻⁶ Prostate cancers that are detected through screening with the serum tumor marker, prostate-specific antigen (PSA), are more likely to be localized to the prostate than

are tumors detected by digital rectal examination.⁷ Observational studies suggest that the treatment of localized prostate cancer by radical prostatectomy better reduces mortality compared with more conservative therapy.^{8,9} Thus, indirect evidence suggests that serologic screening for early prostate cancer may be an effective method of reducing mortality,⁷ although clinical trials are underway to confirm this hypothesis.^{10,11}

The goals of a prostate cancer screening program should include maximizing the number of cancers detected (high sensitivity) and minimizing the number of invasive confirmatory tests. Although PSA provides sufficient sensitivity to diagnose prostate cancer among asymptomatic men,¹² its specificity in the general population has been relatively low because of the high prevalence of irritative and obstructive voiding symptoms resulting from benign prostatic conditions that can also elevate PSA level.^{7,13} In particular, among patients with PSA values between 4 and 10 ng/mL, only 25% are found to have prostate cancer.⁷ Thus, the decision to perform a prostatic biopsy for patients who present with PSA levels below 10 ng/mL is problematic, and physicians may consider other factors associated with increased prostate cancer risk to guide their decision. These factors include age,¹² the presence of a prostatic nodule,¹²

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and a positive family history of prostate cancer.¹⁴ However, for patients with no additional risk factors and whose PSA values are near the cutoff value, the decision to proceed with biopsy remains difficult.¹⁵ More refined PSA-based tests, including PSA density, PSA velocity, and free-to-bound PSA ratios have been proposed, but each test is limited in its ability to distinguish between malignant and benign disease.¹⁶⁻¹⁹ Thus, new serum markers for prostate cancer are desirable.

PSA is a member of the human kallikrein gene family.²⁰ Two other proteins in this family have been described: human kallikrein type 1 and type 2 (hK1 and hK2).²⁰ Production of both PSA and hK2 is stimulated by androgens.²¹⁻²³ The genes that encode these proteins are both located on chromosome 19, and the promoter regions of these genes contain androgen-response elements.^{23,24} These two proteases are closely related and have 80% of their amino acid sequence in common. However, PSA has predominantly chymotrypsin-like protease activity, whereas hK2 has predominantly trypsin-like protease activity.^{25,26} The primary function of the protease activity of PSA is to cleave semen-related proteins,²⁷ but the target for the protease activity of hK2 is unknown.²⁶ It has been proposed that hK2 may be responsible for the activation of the precursor form of PSA to PSA.²⁶

The hK2 protein is predominantly expressed in the prostate epithelial cells and is present in serum, making it a potential marker for prostate cancer.^{20,28} Darson et al²⁹ showed that hK2 expression was less pronounced in benign prostate epithelium than in high grade adenocarcinoma. In contrast, PSA expression was found to be greater in benign epithelium than in prostate cancer cells.²⁹ Finlay et al²⁸ reported consistent serum measurements of hK2 detected by an immunoassay in men with benign and malignant disease and with minimal cross-reactivity with PSA. To date, no studies have reported whether hK2 levels can be used to predict the presence of prostate cancer among patients prescreened with PSA. To answer this question, we conducted a cross-sectional study of 324 men with no history of cancer who presented to one hospital for prostate biopsy because of elevated PSA levels.

PATIENTS AND METHODS

Study Subjects

Patients were drawn from a sample of 404 men who were consecutively referred to the Princess Margaret Hospital between June 1998 and January 1999 because of either PSA values of ≥ 4.0 ng/mL or PSA values between 3 and 4 ng/mL with abnormal digital rectal examination, regardless of the presence of lower urinary tract voiding symptoms. These patients underwent transrectal ultrasonography and needle-core biopsy. Of the 404 patients, 78 were excluded because of either

previous cancer (26 patients), organ transplantation (one patient), prostate biopsy within the last 2 months (four patients), or urinary tract infection within the last 12 months (47 patients). Of the remaining 326 patients, 324 (99%) agreed to provide blood samples for hK2 analysis. Blood samples were collected before clinical prostate examination. Plasma was separated from blood samples and was stored at -70°C . All research was conducted with informed consent and with the approval of the hospital research ethics board.

A urologic history was obtained that included the American Urological Association Symptom Score, which describes the severity of lower urinary tract voiding symptoms.³⁰ The results of digital rectal examination, which was performed by one evaluator (A.T.), were recorded. Systematic sextant ultrasound-guided needle biopsies were performed, with additional directed biopsies as needed, using an 18-gauge spring-loaded biopsy device (Bard Magnum, Murray Hill, New Jersey). The primary end point was the histologic presence of adenocarcinoma of the prostate in the biopsy specimen. Grade was evaluated by the Gleason scoring system.³¹

hK2 and Free-/Total-PSA Analysis

Both free- and total-PSA levels were measured using commercially available kits and performed on the Immulite chemiluminescence immunoassay system (Diagnostic Products Corporation, San Diego, California) according to the manufacturer's recommendations. hK2 levels were measured using a new, time-resolved immunofluorometric assay.³² Briefly, the hK2 assay uses a mouse monoclonal anti-hK2 antibody (coded G586, Hybritech, San Diego, California) that is raised against recombinant hK2, a biotinylated mouse monoclonal detection antibody (coded 8,311, Diagnostic Systems Laboratories, Inc, Webster, Texas), and alkaline phosphatase-labeled streptavidin. The alkaline phosphatase activity was measured by adding the substrate difluorol phosphate, incubating for 10 minutes, and then adding Tb³⁺-EDTA developing solution. The fluorescence was measured on a Cyberfluor 615 Immunoanalyzer (MDS Nordion, Kanata, Ontario, Canada). The hK2 assay has a detection limit of 0.006 ng/mL for both plasma and serum samples and has less than 0.2% cross-reactivity to PSA.³²

Data Analysis

The effect of hK2 levels on prostate cancer risk was examined in two steps. First, we examined whether hK2 levels could predict the presence of prostate cancer after adjusting for other established risk factors with the use of statistical modeling techniques. Second, to determine the clinical utility of hK2 levels, we identified a subgroup of patients with a low probability of having prostate cancer, other than having elevated PSA levels, who nonetheless underwent confirmatory biopsy, and we examined how hK2 levels could alter the probability of detecting prostate cancer.

For the first method, the distributions of the hK2 and the free- and total-PSA levels were compared for patients with and without cancer using nonparametric and parametric tests. The ability of the hK2 level to predict the presence of prostate cancer was examined using univariate and multivariate unconditional logistic regression. The levels of hK2 were considered alone and in combination with free-PSA levels as the hK2:free-PSA ratio. It has been proposed that hK2:free-PSA levels may be a more accurate method of detecting prostate cancer than hK2 levels alone from biologic and clinical observations.^{26,33,34} These two variables were considered as both continuous and categorical variables. The hK2 and hK2:free-PSA ratio values were categorized according to the quartile distribution of the controls. The odds ratio for prostate cancer detection, based on the quartiles of the hK2 and hK2:free-PSA

Table 1. Comparison of PSA and hK2 Measurements Between Patients With Nonmalignant Prostatic Conditions and Prostate Cancer Cases

Variable	Control Groups Compared With Cancer Cases											
	Cancer (n = 165)		All Controls (n = 165)			Benign Prostate Tissue (n = 118)			PIN (n = 47)			
	Mean	Range	Mean	Range	P*	Mean	Range	P*	Mean	Range	P (v Cancer)	P (v Benign)
Total-PSA level, ng/mL	14.5	4-144	10.6	3-82	.10	11.0	3-82	.14	9.6	4-27	.26	.93
Free:total-PSA ratio, %	10.5	2-86	15.9	3-95	.0001	16.1	3-95	.0001	15.6	3-31	.0001	.39
hK2 level, ng/mL	1.18	0.4-11.9	0.53	0.1-4	.0001	0.47	0.6-4	.0001	0.68	0.1-3	.08	.007
hK2:free-PSA ratio, %	1.17	0.06-12	0.62	0.04-2	.0001	0.57	0.04-2	.0001	0.75	0.2-2	.0001	.11

* All *P* values were calculated comparing control groups with cancer cases with the Kruskal-Wallis (nonparametric) test.

ratio values, were determined controlling for age, total-PSA levels, free:total-PSA ratios, digital rectal examination, and the presence of lower urinary tract voiding symptoms. Total serum PSA level was categorized into three groups: 3.0 to 10.0 ng/mL, 10.1 to 20.0 ng/mL, and more than 20.0 ng/mL. Free:total-PSA was divided into quartiles of the controls grouped in descending order, because low free:total-PSA ratios are associated with a higher risk for prostate cancer.¹⁷ Quartiles were used because cutoff values for the free:total-PSA ratio test is highly variable.^{18,35} Digital rectal examination was categorized into three groups: nonpalpable, palpable asymmetric firmness, and palpable nodule. Lower urinary tract symptoms were dichotomized as present or absent. Transrectal ultrasonography results were not considered because the predictive value of this technique has not been well established.^{36,37}

For the second method, we identified a subgroup of patients who had a low probability for prostate cancer, other than abnormal PSA levels, who underwent prostatic biopsy. This subgroup was defined as patients with PSA levels between 3 and 20 ng/mL, nonpalpable nodule on digital rectal examination, and lower urinary tract voiding symptoms. Pre- and posttest likelihood for prostate cancer were determined based on quartile cutoff values for hK2 levels. Because study subjects were sampled based on PSA screening, the computation of sensitivity and specificity for all possible cutoff values with receiver operating characteristics curves³⁸ would have been biased and beyond the scope of this study.

Finally, for patients diagnosed with cancer, mean hK2 levels, hK2:free-PSA ratios, total-PSA levels, and free:total-PSA ratios were compared among different histologic grades. Grading was based on the Gleason scoring system³¹ and was divided into three categories: Gleason scores 2 to 6, 7, and 8 to 10. Stage was not compared because complete staging with radiographic testing was not yet performed.

RESULTS

A total of 324 men underwent transrectal ultrasound-guided needle-core prostatic biopsy. Of the 324 men, 159 (49.1%) were found on biopsy to have adenocarcinoma of the prostate (cases). Of the 165 men with no evidence of cancer (controls), 83 (50%) had normal prostate tissue, 35 (21%) had evidence of inflammation, benign prostatic hyperplasia, or cellular atypia, and 47 (29%) had prostatic intraepithelial neoplasia (PIN). The mean ages of cases and controls were 66.6 and 65.4 years, respectively (*P* = .12).

The hK2 levels, the hK2:free-PSA ratios, and the free:total-PSA ratios were significantly higher in patients with

cancer than in patients with PIN or benign prostate conditions (Table 1). The differences in PSA levels did not reach statistical significance. Only the hK2 measurements were able to discriminate between the presence of benign disease and PIN in this study sample. Among the controls, total-PSA levels were moderately correlated with hK2 levels (Spearman's *r* = 0.42; *P* = .0001). There was no correlation between total-PSA levels and hK2:free-PSA ratios (Spearman's *r* = 0.01; *P* = .87) and between free:total-PSA ratios and hK2 levels (Spearman's *r* = 0.12; *P* = .13).

Quartiles were constructed based on the distribution of the controls. The crude odds ratio for detection of prostate cancer for patients in the highest quartile of hK2 levels compared with patients in the lowest quartile was 5.8 (95% CI, 2.8 to 12.1; *P* = .0001), and there was a clear linear trend in risk for prostate cancer by quartile (Table 2). The crude odds ratio for the presence of prostate cancer for patients in the highest quartile of the hK2:free-PSA ratio was 7.4 (95% CI, 3.58 to 15.1; *P* = .0001) compared with patients in the lowest quartile, and there was also a clear linear trend in risk for prostate cancer by quartile (Table 2). In contrast, only patients who had free:total-PSA ratios in the lowest quartile had a high risk for prostate cancer compared with patients with ratios in the highest quartile (crude odds ratio, 6.73; 95% CI, 3.36 to 13.5; *P* = .0001). Patients with free:total-PSA ratios in the second and third quartiles did not have a significant increase in risk for prostate cancer compared with patients in the highest quartile (Table 2).

The crude odds ratio for the presence of prostate cancer for patients with PSA levels of more than 20 ng/mL was 2.57 (95% CI, 1.16 to 5.72; *P* = .02) compared with patients with PSA levels between 3 and 10 ng/mL. No significant increase in risk for prostate cancer was present for patients with PSA levels between 11 and 20 ng/mL compared with patients with PSA levels between 3 and 10 ng/mL (Table 2). Both abnormal digital rectal examination and the absence of obstructive urinary symptoms were important predictors of prostate cancer in the univariate analysis (Table 2).

Table 2. Univariate Analysis for Each Risk Factor for Prostate Cancer, Including hK2 Measurements, PSA Measurements, Digital Rectal Examination, Lower Urinary Tract Voiding Symptoms, and Age, in Predicting the Presence of Prostate Cancer

Predictor Variable	Crude Odds Ratio	95% CI	P
hK2 level,* ng/mL			
< 0.22	1.00		
0.22-0.38	2.05	0.93-4.53	.08
0.39-0.64	3.63	1.71-7.68	.0008
> 0.64	5.83	2.81-12.1	.0001
hK2:free-PSA† ratios			
< 0.28	1.00		
0.28-0.53	1.15	0.49-2.73	.74
0.54-0.84	2.54	1.17-5.51	.02
> 0.84	7.36	3.58-15.1	.0001
Total-PSA, ng/mL			
3-10	1.00		
11-20	1.10	0.68-1.79	.70
> 20	2.57	1.16-5.72	.02
Free:total-PSA ratios			
> 13.38	1.00		
8.59-13.38	1.30	0.59-2.89	.52
0.06-8.58	1.53	0.70-3.35	.28
< 0.06	6.73	3.36-13.5	.0001
Digital rectal examination			
Nonpalpable	1.00		
Firmness	2.22	1.29-3.84	.004
Nodule	3.31	1.85-5.92	.0001
Urinary symptoms			
Absent	1.00		
Present	0.63	0.40-0.99	.05
Age, continuous	1.02	0.99-1.05	.20

*Test for linear trend (with quartiles assigned ascending values from one to four): Wald $\chi^2 = 26.6$, $P = .0001$; as continuous variable, $P = .0001$.

†Test for linear trend (with quartiles assigned ascending values from one to four): Wald $\chi^2 = 41.6$, $P = .0001$; as continuous variable, $P = .0001$.

In multivariate analysis, both hK2:free-PSA ratios and total-PSA levels were significant predictors for the presence of prostate cancer (Table 3). The hK2:free-PSA ratio generated a high odds ratio for prostate cancer detection (odds ratio, 8.1; 95% CI, 3.73 to 17.4; $P = .0001$) (Table 3). The hK2 level was also predictive for prostate cancer (Table 3). In contrast, among patients with PSA levels of more than 20 ng/mL, the odds ratio was 2.73 (95% CI, 1.09 to 6.86; $P = .03$) compared with patients with PSA levels of less than 10 ng/mL (Table 3). Because the free:total-PSA ratio has been shown to be a better marker for prostate cancer than is total-PSA level,¹⁷ we examined whether hK2 levels were predictive of prostate cancer controlling for free:total-PSA. After controlling for age, digital rectal examination, lower urinary tract voiding symptoms, and free:total-PSA ratio, the hK2 level was predictive for the presence of

prostate cancer. The adjusted odds ratio for having prostate cancer for patients in the second, third, and fourth quartiles of hK2 level compared with patients in the lowest quartile were 2.48 (95% CI, 1.0 to 6.2; $P = .05$), 4.57 (95% CI, 1.9 to 10.9; $P = .0006$), and 10.2 (95% CI, 4.1 to 25.1; $P = .0001$), respectively. From the same analysis, the adjusted odds ratio for having prostate cancer for patients in the third, second, and first quartiles of free:total-PSA level compared with patients in the highest quartile were 1.44 (95% CI, 0.6 to 3.5; $P = .43$), 2.01 (95% CI, 0.83 to 4.84; $P = .12$), and 10.87 (95% CI, 4.8 to 24.8; $P = .0001$), respectively.

Because our study subjects were sampled based on total-PSA values, we used the hK2:free-PSA ratio to determine the pre- and posttest likelihood of prostate cancer for patients at different levels of total-PSA values. We used cutoff values based on the quartile distribution. For patients at the lowest risk for prostate cancer, who had PSA levels of 3 to 20 ng/mL, nonpalpable nodule, and symptoms of urinary tract obstructions, the likelihood for prostate cancer decreased from 34% to 6% if the hK2:free-PSA ratio was in the bottom quartile (Table 4).

Among the 159 patients diagnosed with cancer, 42 (26.4%), 79 (49.7%), and 38 (23.9%) had Gleason scores 2 to 6, 7, and 8 to 10, respectively. Both the hK2 and PSA measurements were correlated with grade at presentation (Table 5). However, the directions of the trends were opposite for hK2 measurements versus the free:total-PSA ratios (Table 5).

DISCUSSION

We found a strong positive association between the serum hK2 level and the risk of prostate cancer among unselected men with elevated PSA levels. In this group, the hK2 measurements performed better or as well as the total-PSA level, the free:total-PSA ratio, or the digital rectal examination. Two small studies have reported on this subject. Kwiatkowski et al³³ showed that, among patients with PSA levels between 4 and 10 ng/mL, those who had prostate cancer had higher hK2:free-PSA ratios than did those who had no evidence of cancer. However, no differences in the levels of serum hK2 were found in that study of 20 cancer patients. Magklara et al³⁴ also showed that hK2:free-PSA ratios were higher in patients with prostate cancer than in those with benign prostatic hyperplasia, but the hK2 level alone was not predictive.

Serum hK2 measurements may help in the selection of patients for prostatic biopsy. The low positive predictive value of PSA has been reported to be as low as 14%.¹⁴ Adjunct serum markers may be more important for patients who present with slightly or moderately elevated PSA values but with no additional risk factors. Among patients with PSA levels between 3 and 20 ng/mL, normal digital

Table 3. Two Multivariate Analyses of hK2 With Free-Total PSA Ratios in Predicting the Presence of Prostate Cancer

Covariate	Multivariate Analysis for hK2 Levels			Multivariate Analysis for hK2:Free-PSA Ratio Levels		
	Odds Ratio	95% CI	P	Odds Ratio	95% CI	P
hK2 level,* ng/mL						
< 0.22	1.00			—	—	—
0.22-0.38	2.10	0.92-4.80	.08			
0.39-0.64	4.03	1.82-8.91	.0006			
> 0.64	6.72	2.90-15.6	.0001			
hK2:free-PSA† ratio						
< 0.28				1.00		
0.28-0.53	—	—	—	1.18	0.48-2.89	.73
0.54-0.84				2.75	1.22-6.20	.01
> 0.84				8.06	3.73-17.4	.0001
Total-PSA, ng/mL						
3-10	1.00			1.00		
11-20	0.92	0.52-1.62	.77	1.30	0.74-2.29	.36
> 20	1.48	0.58-3.77	.41	2.73	1.09-6.86	.03
Digital rectal examination						
Nonpalpable	1.00			1.00		
Firmness	2.86	1.56-5.23	.0007	2.99	1.59-5.65	.0007
Nodule	3.28	1.74-6.20	.0002	3.64	1.88-7.06	.0001
Urinary symptoms						
Absent	1.00			1.00		
Present	0.58	0.35-0.97	.04	0.74	0.44-1.26	.27
Age, continuous	0.99	0.97-1.03	.28	1.01	0.98-1.05	.38

NOTE. The first multivariate analysis considers hK2 levels, whereas the second analysis considers the hK2:free-PSA ratio, because the ratio is a function of the hK2 level.

*Test for linear trend (with quartiles assigned ascending values from one to four): Wald $\chi^2 = 22.9$, $P = .0001$; as continuous variable, $P = .0003$.

†Test for linear trend (with quartiles assigned ascending values from one to four): Wald $\chi^2 = 39.1$, $P = .0001$; as continuous variable, $P = .0001$.

rectal examination, and obstructive urinary symptoms that suggest benign prostatic disease (which represent one third of our population), only 6% were found to have cancer if their hK2:free-PSA ratios were less than 0.28. This value may be low enough that prostate biopsy may be avoided. Other hK2:free-PSA ratio cutoff values do not seem to significantly alter the probability for prostate cancer after considering PSA level, digital rectal examination, and urinary obstructive voiding symptoms. Particularly for patients with a high probability for having prostate cancer because of PSA levels of more than 20 ng/mL, abnormal digital rectal examination, or absence of obstructive urinary symptoms with PSA values of greater than 4 ng/mL, biopsy is clearly indicated because their probability for cancer is 57% and hK2 measurements would not provide any additional diagnostic value.

Both hK2 levels alone and the ratio of hK2 to free-PSA levels were associated with high odds ratios for prostate cancer detection. Future studies are needed before their relative importance can be evaluated. Nonetheless, the validity of the hK2:free-PSA ratio over the hK2 levels alone seems to be consistent with current knowledge regarding the biology of human kallikrein proteins. Bjork et al³⁹ showed that proteins that bind PSA, mainly alpha-1-antichymotrypsin, are synthesized by prostate cancer cells, but not by

benign hyperplastic cells. Thus, lower free-PSA levels would be associated with prostate cancer.³⁵ However, Jung et al¹⁸ showed that asymptomatic chronic inflammatory cells of the prostate also affect alpha-1-antichymotrypsin levels and alter the level of free PSA. In contrast, hK2 seems to be overexpressed by prostate cancer cells but is not expressed by inflammatory cells.^{29,33} Also, Finlay et al²⁸ reported slightly higher hK2 levels among men with benign prostatic hyperplasia than among men with normal prostates. Thus, hK2 and free-PSA levels seem to biologically complement each other.

In our study, patients with PIN had higher hK2 levels than did patients with healthy prostates (mean levels, 0.68 v 0.47 ng/mL, respectively; $P = .009$). In contrast, PSA measurements were not significantly different for patients with PIN and benign prostatic disease. It has been suggested that PIN is a precursor form of prostate cancer.^{40,41} Darson et al²⁹ found an incremental increase in hK2 expression from benign prostate disease to PIN to cancer.

For patients with cancer, both hK2 and PSA measurements were positively associated with grade at presentation. Grade is one of the most important prognostic factors for prostate cancer progression,⁴² and therefore, hK2 levels may play an important role in detecting aggressive prostate cancer. Also, given that serum PSA level at diagnosis is an

Table 4. Pre- and Posttest Likelihood of Prostate Cancer Detection

Risk Group	Pretest Probability for Prostate Cancer		hK2:Free-PSA Ratio Cutoff Value by Quartile Limits		Posttest Probability for Prostate Cancer	
	%	No.	Ratio	No.	%	No.
Low likelihood for the presence of prostate cancer (n = 107) (PSA 3-20 ng/mL and nonpalpable prostatic lesion and lower urinary tract symptoms)	34	36	≤ 0.28	18	6	1
			> 0.28	89	39	35
			≤ 0.53	36	11	4
			> 0.53	71	45	32
			≤ 0.84	64	17	11
High likelihood for the presence of prostate cancer (n = 217) (PSA > 20 ng/mL or palpable prostatic lesion or no lower urinary tract symptoms)	57	123	> 0.84	43	58	25
			≤ 0.28	35	33	12
			> 0.28	182	61	111
			≤ 0.53	73	32	23
			> 0.53	144	69	100
			≤ 0.84	119	42	50
			> 0.84	98	74	73

NOTE. Pre- and posttest likelihood of prostate cancer detection was based on hK2:free-PSA ratio cutoff values from quartile limits, which were based on controls for patients with low and high likelihood for the presence of prostate cancer; this likelihood was based on PSA value, digital rectal examination, and the presence of lower urinary tract voiding symptoms.

important prognostic factor for disease progression,⁴ it is likely that hK2 levels may also be of prognostic importance. To learn whether hK2 levels in addition to PSA contribute to the prognosis of prostate cancer progression will require the examination of how these levels influence stage at presentation and treatment outcomes, with long-term follow-up.

A possible limitation in our study is that a single prostate biopsy set was performed. Among patients who are at risk for prostate cancer because of elevated PSA levels, false-negative biopsies are common and the true prevalence of cancer may be underestimated. Keetch et al⁴³ reported a 19% cancer detection rate among men who had persistently elevated PSA levels at repeat biopsy 1 year after negative biopsy. Prospective follow-up of these patients will be

required to confirm our results and to establish whether elevated hK2 measurements predict cancer at a later date.

Our study did not include patients whose PSA levels were less than 3 ng/mL. It may be that hK2 levels will be found to be most useful for screening the group of patients with PSA levels between 2 and 4 ng/mL. Catalona et al⁴⁴ reported that among men with PSA levels of 2.6 to 4.0 ng/mL, cancer was present in 22%. In our study, eight patients had PSA levels between 3 and 4 ng/mL. Of these eight patients, two were diagnosed with prostate cancer (25%). Furthermore, both of these patients had hK2:free-PSA ratios in the third quartile. Prospective, population-based screening studies are needed to address these issues.

Table 5. Comparison of hK2 and PSA Measurements by Grade and Stage at Presentation (n = 159)

Variable	Grade (Gleason score)						P (overall)
	2-6 (n = 42)		7 (n = 79)		8-10 (n = 38)		
	Mean	Range	Mean	Range	Mean	Range	
hK2 level, ng/mL	0.77	0.04-4.1	0.97	0.05-7.5	2.10	0.2-11.9	.006
hK2:free-PSA ratio, %	0.86	0.06-2.4	1.12	0.1-2.8	1.61	0.3-12.6	.01
Total-PSA level, ng/mL	10.4	3.8-45	12.0	3.9-41	24.3	4.8-143.6	.02
Free:Total-PSA ratio, %	12.6	3.3-28.4	10.4	1.7-85.9	8.6	1.6-20.5	.007

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