

Early detection of prostate cancer in African-American men through use of multiple biomarkers: human kallikrein 2 (hK2), prostate-specific antigen (PSA), and free PSA (fPSA)

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Recent studies have reported enhanced prostate cancer detection in Caucasians with serum human glandular kallikrein 2 (hK2) in combination with total- (tPSA) and free-prostate-specific antigen (fPSA). The purpose of this study is to validate these findings in an African-American patient cohort. A total of 137 African-American men were found by routine screening to have tPSA levels above 2.5 ng/ml or an abnormal digital rectal examination. Sera were drawn prior to biopsy of the prostate and Hybritech[®] PSA, fPSA and hK2 (for research use only, not for use in diagnostic procedures) concentrations were determined on Beckman Coulter's Access[®] immunoanalyzer. These independent variables and the ratios of percent fPSA (%fPSA), hK2/tPSA, hK2/fPSA, and hK2*tPSA/fPSA were compared between cancer and non-cancer groups. In all, 49 of 137 men had prostate cancer. hK2 and its calculated ratios outperformed tPSA on receiver operator characteristic (ROC) analysis, but %fPSA had statistically the highest area under the curve (AUC) at 0.801. When restricting the analysis to only the tPSA range of 4.0–10 ng/ml, hK2/fPSA yielded the highest AUC (0.721). The ratio of hK2/fPSA was also found to increase the positive predictive value (PPV) of the %fPSA ranges less than 10 and 10–25%. %fPSA offered the best performance and highest specificity in prostate cancer detection in African-American males over the entire range of tPSA. hK2/fPSA may offer modest improvement in the tPSA range of 4.0–10 ng/ml. Furthermore, hK2/fPSA can enhance the PPV of low %fPSA values. Therefore, the use of multiple biomarkers may ultimately increase the specificity of prostate cancer screening in African-American men.

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Introduction

Screening for prostate cancer using prostate-specific antigen (PSA), with or without digital rectal examination (DRE), starting at the age of 50 y in all males or earlier in men with certain risk factors, is becoming accepted as standard practice across the globe. Studies in the United

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States,¹ Austria,² and Japan³ have demonstrated a significant beneficial stage migration in cancers detected by screening, which translated into lower prostate cancer mortality rates.^{2,3} The underlying belief is that screening increases the number of localized, curable cancers detected, thus avoiding the morbidity of metastatic disease and eventual mortality.

Prostate cancer screening continues, however, to subject a large number of unaffected men to invasive biopsy procedures to rule out cancer. Methods to improve prostate cancer detection, and thereby further enhance the benefits of screening, have largely focused on changing the threshold PSA value^{4,5} or the use of PSA isoforms, such as free PSA (fPSA) or complexed PSA (cPSA), or their calculated ratios.^{6–8} Human glandular kallikrein 2 (hK2), however, may offer another means for clinicians to identify prostatic adenocarcinoma in its early form. hK2 shares 80% of its amino-acid sequence with PSA. It also is produced mainly in the prostatic epithelium, but at serum concentrations 50–100 times less than PSA. Like PSA, hK2 is influenced by androgen stimulation.⁹ However, hK2 levels are independent of PSA levels.¹⁰ Preliminary research studies following serum hK2 concentrations show that this marker is generally elevated in men with prostate cancer as compared to benign prostatic hypertrophy (BPH).^{11–15}

Certainly, as with any screening program, increasing yields can be obtained by targeting specific populations with a higher incidence of the disease in question.¹⁶ The African-American male population has been shown to have up to twice the age-adjusted incidence and mortality of prostate cancer as compared to Caucasian Americans.^{17–19} The cause of this disparity is not currently known. Any improvement in the detection or treatment of prostate cancer could theoretically offer the most benefit to this particularly vulnerable population.

Prior research has suggested that hK2 performs best in combination with the established tumor markers PSA and fPSA.^{12,20} These studies were carried out on largely Caucasian populations only. Whether these results can be specifically applied to African Americans is unknown. Therefore, this study examines the utility of a panel of biomarkers, including total PSA (tPSA), fPSA, and hK2, for improving the specificity of prostate cancer detection in an entirely African-American patient population.

Methods

We prospectively enrolled all men who presented for free prostate cancer screening at the Louisiana State University Health Sciences Center (LSUHSC) in New Orleans from September 1998 to April 2002 with a total PSA greater than or equal to 2.5 ng/ml or an abnormal digital rectal examination (DRE), as determined by urologists at our institution. Serum was collected prospectively, prior to DRE, and immediately aliquoted and frozen at -80°C . Hybritech[®] PSA, fPSA, and hK2 serum concentrations were determined on Beckman Coulter's Access[®] immunoanalyzer. All patients underwent transrectal ultrasound-guided (TRUS) biopsy by the urologist of their choice, and consented to the release of their biopsy information for the purpose of our study. All protocols for this study were approved by the Institutional Review Board at LSUHSC.

The patients were divided into a cancer and a noncancer group. Using SAS JMP statistical software (Cary, NC, USA), sample means for each serum parameter were compared with the Student's *t*-test; sample medians were evaluated by nonparametric Kruskal–Wallis tests. Receiver operator characteristic curves (ROC) were generated and the areas under the curve (AUC) were computed with Analyze-it v1.50 software (Leeds, England, UK). We then computed ratios between free and total PSA (%fPSA), hK2/tPSA, hK2/fPSA, and hK2*tPSA/fPSA, and performed the same analyses. For all statistical comparisons, significance was assumed at a level at or below 0.05. To evaluate the ability of the serum biomarkers to predict biopsy outcomes, sample means and medians of patients with all TRUS biopsy information available were compared in low *vs* high stage and grade and also for tertiles of percentage of biopsy cores positive.

Finally, based on work previously published by Partin *et al*,¹² we examined whether prostate cancer detection could be enhanced by using all three markers together. Patients were stratified by %fPSA ranges to determine if the positive predictive value could be improved by subsequently utilizing the ratio of hK2/fPSA as well.

Results

A total of 7919 unique individuals were screened through LSUHSC during the study years. In total, 913 men qualified for biopsy, of which 513 self-reported race to be African American. Overall, 137 of these men underwent TRUS biopsy and were able to be evaluated. The mean age of 61.1 y in the cancer group was not significantly different from the noncancer group (59.2 y, $P=0.21$). Table 1 demonstrates the distribution of tPSA values for our patients. In all, 49 were diagnosed with prostate adenocarcinoma, yielding a malignancy rate of 35.8%. A total of 21 of the patients were found to have suspicious DREs; only six were found to harbor cancer. Of note, only two patients in the tPSA range 2.5–4.0 ng/ml had abnormal DREs, neither of whom had positive biopsies.

The mean and median values for all direct and calculated measures are shown in Table 2. For all variables except fPSA concentration and hK2/tPSA, there was statistical significance between the cancer and the noncancer groups. Based on these findings, all further statistical work omitted absolute fPSA concentrations and the hK2/tPSA ratio.

Figures 1a and b demonstrate the performance of the traditional serum markers tPSA and %fPSA along with

Table 1 Prostate biopsy results as a function of total PSA.

PSA range (ng/ml)	Bx result		% with cancer
	Negative	Positive	
<2.5	13	1	7.1
2.5–4.0	25	10	28.6
4.0–10	44	27	38
10–20	4	3	42.9
>20	2	8	80
Total	88	49	35.8

Table 2 Comparison of mean and median values for the various serum parameters in those patients with and without cancer.

	Biopsy result		P-value
	Positive	Negative	
<i>n</i>	49	88	
Mean hK2	0.156	0.064	0.005
SD	0.287	0.067	
Median hK2	0.066	0.051	0.004
Max	1.86	0.482	
Min	0.011	0.001	
Mean tPSA	18.94	5.41	0.038
SD	60.37	4.35	
Median tPSA	5.12	4.44	0.024
Max	420.2	31.99	
Min	2.34	0.45	
Mean fPSA	1.21	0.831	0.2
SD	2.64	0.67	
Median fPSA	0.525	0.65	0.19
Max	17.71	4.77	
Min	0.15	0.08	
Mean %fPSA	0.099	0.165	<0.0001
SD	0.044	0.068	
Median %fPSA	0.089	0.162	<0.0001
Max	0.207	0.374	
Min	0.029	0.031	
Mean hK2/fPSA	0.172	0.108	0.013
SD	0.139	0.143	
Median hK2/fPSA	0.137	0.07	0.0001
Max	0.714	1.138	
Min	0.008	0.005	
Mean hK2/tPSA	0.016	0.016	0.9989
SD	0.015	0.02	
Median hK2/tPSA	0.012	0.01	0.28
Max	0.084	0.098	
Min	0.001	0.001	
Mean hK2*tPSA/fPSA	2.35	0.444	0.006
SD	6.4	0.41	
Median hK2*tPSA/fPSA	0.852	0.321	<0.0001
Max	44.14	2.28	
Min	0.056	0.006	

hK2: human glandular kallikrein 2; tPSA: total prostate-specific antigen; fPSA: free PSA; %fPSA: percent free PSA; SD: standard deviation. ¹²⁵IhK2, tPSA, fPSA, and hK2*tPSA/fPSA values are given in units of ng/ml.

hK2 and hK2/fPSA for the whole population of this study. While the AUC for hK2 alone (0.647) and hK2/fPSA (0.699) was significantly higher than that of tPSA (AUC = 0.617), %fPSA performed best (AUC = 0.801, pairwise $P < 0.05$). The calculated ratio hK2*tPSA/fPSA (not shown) performed equal to hK2/fPSA (AUC 0.766, $P = 0.24$).

tPSA has the least discriminatory power at levels below 10 ng/ml. Therefore, we examined the biomarker performance below this limit, where screening could most be enhanced. In all, 106 of the 137 patients in the study had tPSA values between 2.5 and 10 ng/ml. No change in the AUC values reported above was found. However, not all urologists today use 2.5 ng/ml as their cutoff for biopsy. When restricting our analysis to only those with tPSA between 4.0 and 10 ng/ml, a different picture emerges (see Figure 2a and b). %fPSA again

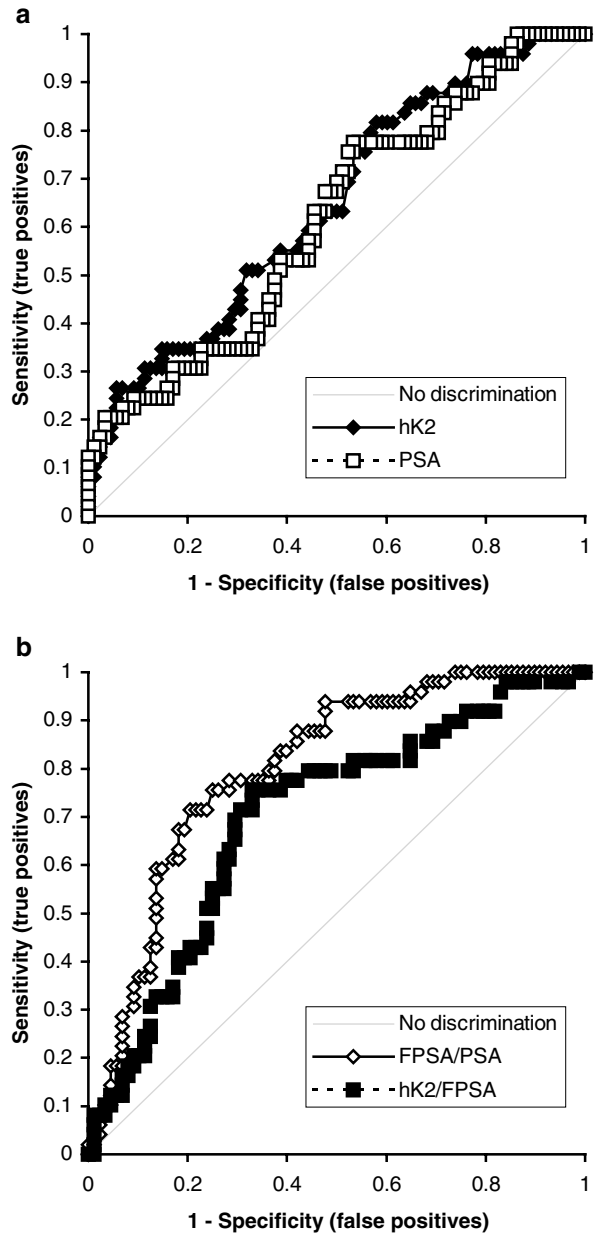


Figure 1 For all 137 patients over the entire range of total PSA values: (a) tPSA (AUC = 0.617) and hK2 (AUC = 0.647). (b) %fPSA (AUC = 0.801) and hK2/fPSA (AUC = 0.699).

outperformed the simple measures of hK2 and tPSA (AUC = 0.713 vs 0.579 and 0.612, respectively; pairwise $P < 0.05$). However, the ratio of hK2/fPSA gave the best discriminatory power of all the tests (AUC = 0.721, $P < 0.05$).

Table 3 depicts the more clinically relevant measures of specificity at given levels of sensitivity. At all levels of sensitivity shown, the ratio of hK2/fPSA has higher specificity than tPSA alone for all patients in the study. For the 71 patients with tPSA of 4.0–10 ng/ml, hK2/fPSA again consistently yields higher specificity than tPSA at various sensitivities. Overall, though, %fPSA delivers significantly higher specificities for both the entire tPSA range and what has been called the diagnostic ‘gray zone’ from 4.0 to 10 ng/ml.

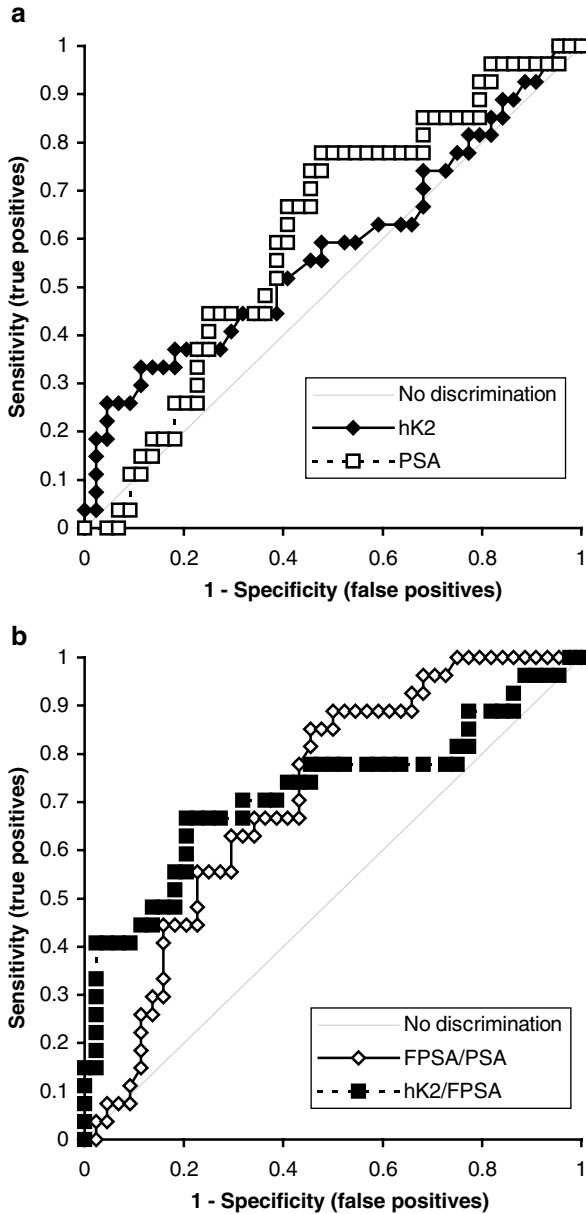


Figure 2 For the 71 patients with tPSA between 4.0 and 10 ng/ml only. (a) tPSA (AUC=0.612) and hK2 (AUC=0.579). (b) %fPSA (AUC=0.713) and hK2/fPSA (AUC=0.721).

Table 4 demonstrates the combined use of hK2/fPSA with %fPSA in the range of PSA 2.5–10 ng/ml. When the %fPSA is less than 10%, an hK2/fPSA value greater than 0.10 carries a 73.1% positive predictive value for cancer. Similarly, for %fPSA between 10 and 25%, 25.4% of our patients were diagnosed with cancer. However, if the hK2/fPSA ratio was greater than 0.12 in this %fPSA range, 47% had cancer versus 17% if below this threshold ($P=0.027$).

Of the 49 men with cancer, 40 had complete clinical stage and grade information available. From these men, an average 9.8 transrectal biopsy samples were taken with an average 3.4 positive for cancer. In total, 23 had less than one-third of their cores positive. The average Gleason sum was a 3+3 pattern, with only two patients having primary Gleason score 4 patterns. In all,

Table 3 Threshold levels for cancer detection for each serum variable at various levels of sensitivity

	% Sensitivity		
	85	90	95
<i>For all tPSA values (n = 137)</i>			
tPSA			
Cutoff (ng/ml)	3.26	2.87	2.53
Specificity (%)	28.4	19.3	14.8
hK2			
Cutoff (ng/ml)	0.033	0.025	0.024
Specificity (%)	35.2	23.9	22.7
%fPSA			
Cutoff (%)	15.4	16	19.4
Specificity (%)	58.0	52.3	35.2
hK2/fPSA			
Cutoff	0.046	0.035	0.026
Specificity (%)	35.2	25.0	17.0
<i>For tPSA between 4.0 and 10 ng/ml (n = 71)</i>			
tPSA			
Cutoff (ng/ml)	4.48	4.2	4.02
Specificity (%)	18.5	11.1	0
hK2			
Cutoff (ng/ml)	0.03	0.02	0.11
Specificity (%)	18.2	13.6	6.8
%fPSA			
Cutoff (%)	15.5	19.1	20
Specificity (%)	54.5	34.1	31.8
hK2/fPSA			
Cutoff	0.035	0.027	0.021
Specificity (%)	22.7	13.6	11.4

19 were classified as clinical T1c, three as cT2a/b, 15 cT2c, and only one was staged as cT3. When looking only at the 40 patients with cancer and complete biopsy data, none of our variables were statistically different for low vs high Gleason sum (less than 7 vs 7 or greater) or clinical stage (T1c vs T2 or greater, data not shown). Mean values for hK2, tPSA, and hK2*tPSA/fPSA increased significantly in the tertiles from less than one-third biopsy cores positive to greater than two-thirds ($P < 0.012$ for each, data not shown). No differences were seen when looking at the sample medians for these parameters, however.

Discussion

PSA is a member of the human tissue kallikrein gene family, which has been mapped to chromosome 19q13.2-13.4.²¹ PSA is produced almost exclusively by the prostatic epithelium, to be released in the prostatic ducts for participation in the liquefaction of the ejaculatory coagulum.⁹ PSA has been found to have tumor suppressor functions, such as the induction of apoptosis, as well as mitogenic properties, such as the liberation of insulin-like growth factor-1 or the activation of transforming growth factor- β .²² Therefore, PSA may play a central role in the carcinogenic process.

Table 4 Practical use of %fPSA and hK2 in the 2.5–10 ng/ml total PSA range

%fPSA range	n	% CaP	P*	hK2/fPSA Range	n	%CaP	P**
<10	31	61	0.0013	<0.10	5	0	0.0093
10–25	71	25		≥0.10	26	73	
				<0.12	52	17	0.0271
				≥0.12	19	47	
>25	4	0		NA			

*Fisher exact test by Analyse-it, with >25% fPSA group left out.

**Fisher exact test by Analyse-it.

NA = not applicable.

Another prostatic secretory product, hK2, is highly homologous to PSA. One of its major functions in the prostate may be to convert proPSA to the active PSA enzyme, but it may also participate in the autoactivation of hK2.⁹ Through its trypsin-like enzyme activity, hK2 may also play a role in the proteolytic cascade that allows for prostate cancer invasion and eventual metastasis.²²

Early attempts to detect hK2 in sera were met with difficulty due to concentrations often lying below the detection threshold.¹⁰ Therefore, initial studies were performed at the tissue level. Darson *et al*²³ demonstrated increased immunohistochemical staining for hK2 as one progressed from benign glands to prostatic intraepithelial neoplasia to cancer. PSA showed a decreasing staining pattern. This correlated well with a later study showing increased mRNA expression for hK2 in cancer cells compared to benign glands, whereas PSA mRNA had higher expression in the BPH tissue.²⁴ However, Stamey *et al*²⁵ found no difference in PSA or hK2 gene expression when looking at BPH tissue *vs* Gleason grade 4/5 prostatic adenocarcinoma. Magklara *et al*²⁶ found decreased hK2 concentrations with cancer *vs* benign tissue in radical prostatectomy specimens; PSA concentrations were decreased in cancer as well, but to a greater extent.

Better serum assays soon followed, and early reports have shown some benefit to the measurement of hK2 serum concentrations, rather than relying on tissue specimens. Kwiatkowski *et al*¹¹ found that the ratio of hK2/fPSA, not hK2 alone, improved cancer detection in their group of patients with tPSA values between 4 and 10 ng/ml and lower urinary tract symptoms (LUTS). They surmised that due to the independence of hK2 and fPSA concentrations—fPSA being inversely related to cancer while hK2 was directly related—discriminatory power was superior using the calculated ratio. While Magklara *et al*²⁰ found no difference in hK2 concentrations between a cancer group and a BPH group, they showed that men with tPSA concentrations between 2.5 and 4.5 ng/ml were at substantially higher risk if their hK2/fPSA ratio was elevated as well. These conclusions were echoed by a more recent report by Scorilas *et al*.²⁷ In a Swedish study of a screening population referred for biopsy, hK2 levels were significantly higher in the cancer group.¹⁴ In this study, the inclusive ratio of hK2*tPSA/fPSA was shown to have the best discriminatory effect, but not statistically better than %fPSA. In men with tPSA levels between 3 and 20 ng/ml, a nonsuspicious DRE, and LUTS suggestive of BPH, Nam *et al*¹⁵ found that the lowest quartile of hK2/fPSA could be spared biopsy, as the risk of cancer was only 6%.

Our results demonstrate that the use of ratio of hK2/fPSA performed superiorly to the individual use of tPSA or hK2 in a population of African-American males. However, the %fPSA had the best discriminatory power over the entire range of tPSA. When limiting the discussion only to those with tPSA values between 4.0 and 10 ng/ml, hK2/fPSA offered the most statistical power in detecting prostate cancer, as measured by the AUC on ROC analysis. From the clinical standpoint, %fPSA had the highest specificity of all the markers at a screening sensitivity of 95%, as shown in Table 3, regardless of the chosen limits on tPSA.

The combined use of hK2/fPSA in incremental ranges of %fPSA could enhance the positive predictive value of prostate cancer screening. Partin *et al*¹² examined the use of hK2/fPSA combined with %fPSA ranges for tPSA values of 2.5–10 ng/ml. For instance, for patients with tPSA between 4.0 and 10 ng/ml and %fPSA values between 10 and 25%, there was 50% risk of prostate adenocarcinoma when the hK2/fPSA ratio was greater than 0.18 compared to only a 15% risk if below this threshold. The racial composition of this study group was 93% Caucasian. In our population, using a threshold hK2/fPSA value of 0.12 increased the positive predictive value of %fPSA between 10 and 25% from a cancer detection rate of 25% to 47% (see Table 4). Similarly, the use of a cutoff of hK2/fPSA less than 0.10 excluded cancer in the high-risk group of patients with %fPSA less than 10%. In practice, perhaps a screening panel of tumor biomarkers could be drawn and a patient's cancer risk stratified in a manner such as in Table 4. On the other hand, perhaps hK2/fPSA levels could be used to help guide urology specialists in the follow-up of patients previously found to have negative biopsies. If their %fPSA still falls in the higher-risk category of less than 25%, the hK2/fPSA level might be better able to influence the decision to repeat biopsy. Obviously, our work does not specifically address this issue, but it does bring up this interesting point for further research.

In our population, hK2 or its ratios with tPSA and/or fPSA was not able to discern low- from high-grade disease on biopsy or to predict clinical stage. This stands in distinction from prior work in Caucasians examining postprostatectomy specimens.^{28,29} Pathologic data from prostatectomy specimens should be considered the gold standard however; biopsy information does not always correlate well with the final pathologic result.

An incidental finding of this study was a significant cancer detection rate among men with tPSA between 2.5 and 4.0 ng/ml (Table 1). This correlates well with prior studies showing 22–27% prostate cancer rates at these low tPSA levels.^{4,5} Of our patients in this range, only one

had a Gleason score less than 6; however, he had 4 of 6 scores positive. As none of these patients subsequently found to have cancer on biopsy had abnormal rectal examinations, they represent cancers that would otherwise have gone undetected using a tPSA threshold of 4.0 ng/ml.

Conclusions

hK2 serum concentrations in relation to free PSA offer some advantage over tPSA alone in prostate cancer detection, both for what has been called the diagnostic 'gray zone' of tPSA 4.0–10 ng/ml and for all tPSA values, in this African-American patient cohort. %fPSA continues to offer the highest level of specificity at given levels of sensitivity, however. Future directions of hK2 research may employ the combination of %fPSA levels with the ratio hK2/fPSA to better discern those with and without prostate cancer.

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