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HUMAN GLANDULAR KALLIKREIN 2: A POTENTIAL SERUM MARKER FOR PREDICTING THE ORGAN CONFINED VERSUS NONORGAN CONFINED GROWTH OF PROSTATE CANCER

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ABSTRACT

Purpose: We measured serum levels of human glandular kallikrein 2 (hK2) in patients with prostate cancer treated with radical retropubic prostatectomy for clinically localized prostate cancer to determine whether preoperative hK2 levels discriminate stage pT2a/b from pathological stage T3a or greater cancer. This finding would help to predict preoperatively the organ confined versus nonorgan confined growth of prostate cancer.

Materials and Methods: A total of 68 consecutive men underwent radical retropubic prostatectomy for clinically localized prostate cancer. Serum was obtained 1 day preoperatively before prostatic manipulation. hK2, and total and free prostate specific antigen (PSA) were measured using immunofluorometric assays. Mean, median and range of hK2, total and free PSA, and the ratio of free-to-total PSA (percent free PSA) were calculated. Each analyte or combination of analytes was evaluated to determine whether it significantly contributed to enhance the discrimination of organ confined from nonorgan confined cancer. We calculated the statistical significance of observed differences using the Mann-Whitney U and Kruskal-Wallis tests. Sensitivity and specificity calculations were performed for hK2, total PSA and the algorithm, (hK2) × (total PSA/free PSA) in addition to receiver operating characteristics curves and the respective areas under the curves. Multivariate logistic regression analysis was done for hK2, and total and free PSA

Results: Disease was organ and nonorgan (extraprostatic extension) confined in 38 and 30 men, respectively. In organ confined cancer mean hK2 was significantly lower than in nonorgan confined cancer (0.09 ng./ml., range less than 0.03 to 0.23 versus 0.30, range 0.04 to 0.94, $p < 0.0001$). In addition, there was significantly higher free and total but not percent free PSA in nonorgan than in organ confined cases. There were also statistically significant differences in hK2, free PSA and total PSA at each pathological disease stage ($p < 0.001$, < 0.01 and < 0.05, respectively). Sensitivity for detecting organ confined disease was 37% at 100% specificity (correct identification of all nonorgan confined cancer) using hK2 measurements compared with a sensitivity of 14% for total PSA. At a specificity of 95%, sensitivity was 40% for hK2 versus 23% for total PSA, which was a statistically significant gain in sensitivity ($p < 0.05$). Receiver operating characteristics curves demonstrated that hK2 had the largest area under the curve, followed by the algorithm, (hK2) × (total PSA/free PSA), and total PSA (0.76, 0.75 and 0.72, respectively). However, none of area under the curve differences was statistically significant.

Conclusions: Compared with total and free PSA hK2 testing improved the preoperative evaluation of patients who underwent radical retropubic prostatectomy due to the superior discrimination of organ from nonorgan confined cancer.

KEY WORDS: prostate; kallikrein II; prostate-specific antigen; prostatic neoplasms; staging, tumor

Prostate cancer is the most commonly diagnosed cancer in men. Death rates for prostate cancer are second only to those for lung neoplasms.[1] With its introduction into clinical use prostate specific antigen (PSA) emerged as the most important tumor marker in the field of urology.[2–5] The most valuable application of PSA involves postoperative followup after radical prostatectomy when, due to organ specificity sufficient for all practical purposes,[6] evidence of recurrent disease may be based only on the recurrence of PSA in serum.[6–11]

Despite limitations due to a lack of sensitivity and specificity for prostate cancer,[5,9,12,13] PSA testing is also established for diagnosing prostate cancer. The lack of sensitivity and specificity led to the creation of parameters based on PSA to enhance clinical usefulness, such as PSA velocity,[14] PSA density,[15] transition zone PSA densitiy,[16] age specific PSA range[17] and the ratio of free-to-total PSA.[18–20] The application of PSA for preoperatively staging of prostate cancer has demonstrated that serum PSA correlates with tumor volume, and increasing clinical and pathological stage[7,9,13]. However, in an individual single PSA measurements are not specific enough to permit the precise prediction of the final

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***Editor's Note*: This article is the fifth of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 1540 and 1541.**

pathological stage.[11, 12] The most effective approach for treating prostate cancer may be initiated when the tumor is still organ confined. Information may be obtained from the results of systematic sextant biopsies. However, a serum marker for more accurately staging prostatic lesions would provide important and more easily available new information.

Recently the new serum marker of prostatic origin introduction human glandular kallikrein 2 (hK2), a protein closely related to the enzymatic family of so-called serine proteases, emerged as a potential marker for prostate tissue of benign and malignant differentiation.[21–23] The gene for hK2 is 80% homologous to the PSA gene. Recent studies indicated that hK2 and PSA messenger RNA are present exclusively in prostatic epithelium.[24–26] PSA and hK2 also share the feature of androgen controlled expression.[25, 27] Furthermore, hK2 is the enzyme that cleaves pro-PSA and, thus, activates PSA into its enzymatically active form.[28] The less common term, hk3 for PSA, emphasizes the association of these enzymes.

Biochemically the close homology of each protein made it necessary to design monoclonal antibodies that may not cross-react between hK2 and PSA. Investigations have developed monoclonal antibodies,[29] mapped the epitopes of hK2 and PSA,[30] verified the cross-reactivity of anti-PSA antibodies against hK2[31] and created immunoassays for hK2 of various designs.[29, 32] Clinically the serum concentration of hK2 has been used to improve the detection rate of prostate cancer in patients with a total PSA of 4 to 10 ng./ml.[32] In addition, the cytoplasmic expression of hK2 and PSA has been measured in radical prostatectomy specimens.[22]

In this study we focused on the serum concentration of hK2 in 68 men who underwent radical prostatectomy to evaluate whether it differs in organ and nonorgan confined prostate cancer. Since PSA does not reliably predict organ confined cancer in an individual,[11, 12] this capability may be an important feature of this new serum marker for the preoperative biochemical staging of prostatic adenocarcinoma.

MATERIALS AND METHODS

Patient selection and evaluation. Serum samples of 68 men scheduled for radical retropubic prostatectomy for clinically localized prostate cancer were collected 1 day before surgery. No patient received hormonal treatment preoperatively. Serum was collected before any manipulation of the prostate and stored at −80C until analysis. Mean overall patient age was 62.0 years. Mean and median age of those with organ confined cancer was 62.1 and 62.0 years, respectively, which was not significantly different from that of men with nonorgan confined disease. The prostate was prepared according to the Stanford protocol[33] with a 3 mm. step section technique. The Gleason system was used for histological grading,[34] and staging was done according to the TNM classification.

Detection of hK2: second generation assay (set 1). To detect hK2 we used a second generation assay in 16 patients, involving a 3-step immunofluorome-tric assay previously described.[35] Briefly the 3 monoclonal antibodies, mAb 2H11, 10 and 36, which do not cross react with hK2, were given in excess to prevent free and total PSA from reacting in further reaction steps (PSA blocking). Another antibody, mAb H50, was is added that only reacts with hK2 because the corresponding epitope on PSA was blocked in the initial assay step and bound to the streptavidin coated microtitration well. All free and ACT PSA was removed by washing. In step 3 a europium (Eu) labeled antibody, mAb H117, reacted with the immobilized hK2. Eu forms a fluorescent chelate proportional to the amount of hK2. Immunological cross-reaction measured with recombinant PSA was less than 0.1%. The analytical detection limit, defined by measuring +3 standard deviations (SD) of the signal imprecision of the 0 calibrator, was 0.01 ng./ml. The functional detection limit was 0.05 ng./ml., defined as the level at which the interassay coefficient of variation was less than 20%.

Detection of hK2: third generation assay (set 2). To detect hK2 we also used a third generation assay in 52 patients. The third generation hK2 assay was improved by adding an additional antibody, mAb 2E9, to the PSA blocking procedure. The catcher antibody 6H10 replaced the former catcher mAb H50, which became the tracer antibody and replaced H117. H117 was eliminated from the assay. Improved performance resulted in a better analytical detection limit of 0.01 ng./ml., a functional detection limit of 0.03 ng./ml. and an insignificant cross-reactivity to PSA of less than 0.01%.

Detection of total, free and percent free PSA. To detect total and free PSA we performed the DELFIA ProStatus Dual* PSA-total/free assay. The assay uses a sandwich based technique. In step 1 free and total PSA is equimolar bound to a solid phase anti-total PSA antibody. Subsequently Eu labeled antibodies are bound to an antigenic site accessible only in free PSA molecules. Simultaneously samarium labeled antibodies are bound to antigenic sites accessible to free and total PSA. Each lanthanide formed fluorescent chelates in proportion to the amount of free (Eu only) and total (Eu plus samarium) PSA. We calculated the ratio of free-to-total PSA or percent free PSA using each result.

Algorithm using hK2, and total and free PSA. We evaluated 3 algorithms combining hK2 and PSA to achieve more information than provided by each analyte only. The algorithms were hK2 × (total PSA/free PSA), hK2/total PSA and hK2/free PSA. The initial algorithm, hK2 × (total PSA/free PSA), was the most powerful for discriminating organ from nonorgan confined cancer. Thus, we used this algorithm for further analysis and abandoned the latter 2.

Study design and statistics. We compared the mean and median of hK2 in organ and nonorgan confined disease in the 16 patients evaluated by the second generation assay to those of the 52 evaluated by the third generation assay. All hK2 concentrations below the functional detection limit of the second and third generation assays of 0.05 and 0.03 ng./ml. were rated at 0.049 and 0.029 ng./ml., respectively. This manipulation proved necessary to avoid introducing any unsuitable distinction between the men with nonorgan and organ confined cancer in whom hK2 was less than 0.03 and 0.05 ng./ml., respectively (table 1). We calculated the significance of the eventual differences in organ and nonorgan confined disease in each sample set using the Mann-Whitney U test with $p = 0.05$ or less considered significant. Box plots were constructed to visualize the concentration of hK2 and total PSA in organ and nonorgan confined cancer (fig. 1). Table 1 shows the results of hK2 detection, which allowed us

* Perkin Elmer Life Sciences, Wellesley, Massachusetts

TABLE 1. *hK2 in organ and nonorgan confined cancer*

Assay Generation	No. Pts.	Mean/Median Overall (ng./ml.)	Mean/Median Organ Confined (ng./ml.)	Mean/Median Nonorgan Confined (ng./ml.)	p Value (Mann-Whitney U test)
1	16	0.12/0.10	0.07/0.05	0.19/0.14	0.007
2	52	0.18/0.09	0.09/0.07	0.30/0.15	0.0043
Total No.	68				

to summarize the data in a single data set and perform subsequent evaluations.

For each analyte (hK2, and total and free PSA), and for percent free PSA and the hK2 × (total PSA/free PSA) algorithm we calculated the mean, median, range and standard error (SE) in organ and nonorgan confined disease. We determined statistical significance using the Mann-Whitney U test (table 2). Further analysis included the calculation of the mean, median and range per organ confined pathological stages pT2a and pT2b, each nonorgan confined pathological stage T3a or greater and lymph node positive disease separately for hK2 and total PSA (tables 3 and 4). Box plots were constructed to show the concentration of hK2 and total PSA according to pathological disease stage. We performed the Kruskal-Wallis test to determine statistical significance (fig. 2). Calculation of the odds and crude odds ratios was done using multivariate logistic regression analysis to evaluate the ability of hK2, and total and free PSA to predict stage pT2 disease (table 5). We determined the sensitivity and predefined specificity, and specificity at predefined sensitivity for hK2, total PSA and the algorithm. Furthermore, receiver operating characteristics (ROC) curves were designed, and we calculated the area under the curve for hK2, the algorithm and total PSA.

We also analyzed mean Gleason score in organ versus nonorgan confined cancer. We noted a statistically significant difference in organ and nonorgan confined disease. Thus, after excluding from study 3 of our 68 patients due to lymph node metastasis, we calculated mean hK2 in 65 according to Gleason grade 3+3 or less versus 3+4 and 4+3 or greater disease in organ and nonorgan confined cancer (table 6).

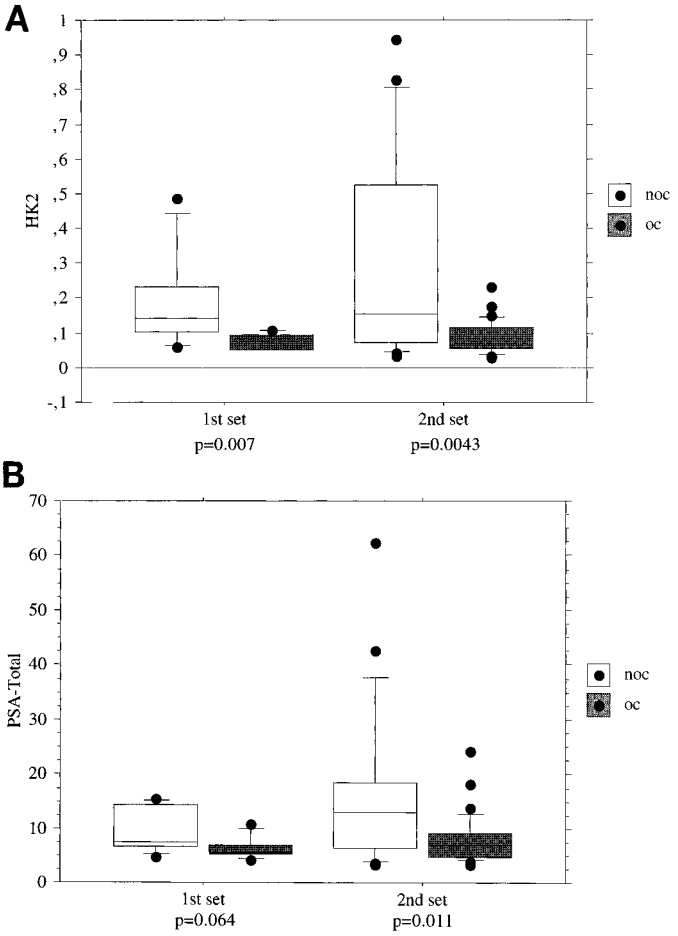

FIG. 1. Box plots of organ confined (*oc*) and nonorgan confined (*noc*) cancer in sets 1 and 2. *A*, hK2. *B*, total PSA.

RESULTS

Of the 68 patients who underwent radical retropubic prostatectomy 38 had organ confined cancer. Pathology evaluation in 30 men revealed nonorgan confined cancer. In 3 men radical retropubic prostatectomy was aborted due to lymph node metastasis. Gleason grade was 3+3 or less in 30 cases, 3+4 in 29 and 4+3 or greater in 6. There was a slight but significant difference in mean Gleason score in organ versus nonorgan confined disease (6.1 versus 6.6, $p < 0.01$). Table 1 shows the mean, median and Mann-Whitney U test results of hK2 determination in organ and nonorgan confined cancer in 16 patients evaluated by the second and 52 by the third generation assays.

In 16 patients the second generation assay detected hK2 at levels of 0.05 ng./ml. or greater, that is above the detection limit, in 4 of the 9 (44.5%) with organ and in all 7 (100%) with nonorgan confined disease. Table 1 and figure 1, *A* show that mean hK2 was significantly lower in organ than in nonorgan confined cancer (0.07 versus 0.19 ng./ml., $p = 0.007$). In contrast, mean total PSA in nonorgan confined cancer was only significantly different from organ confined disease at the 10% but not at the 5% level, as we defined (9.87 ng./ml., range 4.75 to 15.2, $p = 0.064$ and 6.26 ng./ml., range 4.16 to 10.7, respectively, fig. 1, *B*).

In 52 patients the third generation assay detected hK2 at 0.03 ng./ml. or greater, that is above the detection limit, in 27 of 29 (93%) with organ and in all 23 (100%) with nonorgan confined cancer. Table 1 and figure 1, *A* show that mean hK2 was significantly lower in organ than in nonorgan confined cancer (0.09 versus 0.30 ng./ml., $p = 0.0043$). In this group mean total PSA in nonorgan confined cancer was significantly higher than in organ confined disease (16.3 ng./ml., range 3.43 to 62.3 versus 7.88, range 3.34 to 24.1, $p = 0.011$, fig. 1, *B*).

Based on these results we also tested whether any combination of the measured levels of hK2, total and free PSA in serum would further enhance the discrimination of the 38 men with organ confined cancer from the 30 with nonorgan confined disease. Hence, table 2 shows the median, mean and analysis of statistically significant differences by the Mann-Whitney U test in the 2 sets using the hK2 × (total PSA/free PSA) algorithm, free PSA, percent free PSA, hK2 and total PSA. As with hK2, the algorithm, and total and free PSA but not percent free PSA were significantly different in the 2 patient sets (table 2). However, no single analyte or combination of analytes in this data set contributed significant discriminative power in addition to that provided by hK2. This finding may mainly be due to the close correlation of hK2 with total and free PSA, and the close correlation of free with total PSA ($r = 0.79$, 0.87 and 0.88, respectively).

In addition, we created a complete set of data based on hK2 data according to pathological stage (table 3 and fig. 2, *A*) as well as corresponding data on total PSA (table 4 and fig. 2, *B*). These data show a stepwise elevation in serum hK2 in parallel with the stepwise increase in pathological disease stage. Furthermore, there were statistically highly significant differences in hK2 at each pathological stage (Kruskal-Wallis test $p < 0.001$). Our analysis also revealed significant differences at each pathological stage in free and total PSA ($p < 0.01$ and $p < 0.05$, respectively) (data not shown). Any further comparison among pathological stages was of limited value due to the relatively small number of patients at each pathological stage but also because 7 of 38 men with organ confined disease had an hK2 concentration below the detection limit of the assay.

Multivariate logistic regression analysis, and the calculation of the odds and crude odds ratios for each analyte (hK2, and total and free PSA) for predicting stage pT2 disease showed that the crude odds ratio for the separate analysis of hK2 was superior to that of free and total PSA. An increase

TABLE 2. *Results of evaluated analytes and algorithm for discriminating organ from nonorgan confined cancers*

	Mean/Median Overall	Mean/Median Organ Confined	Mean/Median Nonorgan Confined	p Value (Mann-Whitney U test)
hK2 (ng./ml.)	0.18/0.10	0.08/0.07	0.30/0.15	<0.0001
hK2 × total/free PSA	1.54/0.83	0.93/0.65	2.31/1.26	0.0005
Total PSA (ng./ml.)	10.73/7.39	7.50/6.43	14.81/12.30	0.0023
Free PSA (ng./ml.)	1.37/0.89	0.86/0.81	2.03/1.25	0.0037
% Free PSA	13.10/11.63	12.51/11.52	13.85/11.97	0.6745

TABLE 3. *Distribution of hK2 by pathological stage and lymph node status*

Stage	Mean (ng./ml.)	Median (ng./ml.)	SE	No. Pts.	Min. (ng./ml.)	Max. (ng./ml.)
pT2a	0.06	0.05	0.01	5	<0.03	0.11
pT2b	0.09	0.07	0.01	33	<0.03	0.23
Organ confined:	0.08	0.07	0.01	38	<0.03	0.23
pT3a	0.24	0.12	0.06	21	0.04	0.94
pT3b	0.14	0.14	0.00	2	0.14	0.14
pT4a	0.52	0.55	0.15	4	0.13	0.83
Lymph node pos.	0.47	0.52	0.05	3	0.37	0.53
Nonorgan confined	0.30	0.15	0.05	30	0.04	0.94
Overall	0.18	0.10	0.03	68	<0.03	0.94

TABLE 4. *Distribution of total PSA by pathological stage and lymph node status*

Stage	Mean (ng./ml.)	Median (ng./ml.)	SE	No. Pts.	Min. (ng./ml.)	Max. (ng./ml.)
pT2a	6.63	7.47	1.11	5	3.93	9.54
pT2b	7.64	6.29	0.76	33	3.34	24.10
Organ confined:	7.50	6.43	0.67	38	3.34	24.10
pT3a	12.57	10.30	2.25	21	3.43	42.50
pT3b	10.00	10.00	5.24	2	4.76	15.24
pT4a	25.33	15.75	12.49	4	7.53	62.30
Lymph node pos.	19.67	18.10	3.69	3	14.20	26.70
Nonorgan confined	14.81	12.30	2.35	30	3.43	62.30
Overall	10.73	7.39	1.18	68	3.34	62.30

in hK2 by 1 SD (0.2 ng./ml.) increased the likelihood of nonorgan confined cancers by a factor of 14.5 (95% confidence interval [CI] 1.94 to 109). Calculation of the odds ratio of each analyte combined with the other 2 also showed that hK2 was superior to free and total PSA. However, the 95% CI demonstrated that this result was not significant for all 3 analytes. Table 5 shows complete data.

We further evaluated the diagnostic performance of each analyte and combination of analytes by calculating the area under the curve using ROC (fig. 3). hK2 had the largest area under the curve (0.76), while that of the algorithm and total PSA was 0.75 and 0.72, respectively. However, none of the differences in the area under the curves was statistically significant.

There was a statistically significant increase in sensitivity for detecting organ confined disease at 100% specificity, that is correct identification of all cases of nonorgan confined cancer using hK2 versus total PSA (37% versus 14%, $p < 0.05$). At a specificity of 95%, sensitivity was 40% for hK2 but 23% for total PSA ($p < 0.05$). In addition, we also investigated whether there was any correlation of Gleason grade with hK2 in organ and nonorgan confined cancer (table 6). Median hK2 ranged from 0.06 to 0.08 ng./ml. in all men with organ confined cancer of Gleason grades 3+3 or less, 3+4 and 4+3 or greater. Median level in nonorgan confined disease of Gleason grades 3+3 or less and 3+4 were slightly higher (0.11 and 0.12 ng./ml., respectively). However, the median level was much higher (0.62 ng./ml.) in nonorgan confined cancer of Gleason grades 4+3 or greater, although the range of hK2 was wide in all 3 Gleason groups of nonorgan confined disease.

DISCUSSION

The detection of PSA in serum in its complexed and noncomplexed forms is an established part of the pre-therapeutic and post-therapeutic evaluation of prostate cancer. After the diagnosis of prostate cancer is histologically confirmed and evaluation of the patient suggests a clinically localized disease stage, radical prostatectomy is the gold standard of treatment applied with curative intent. However, several studies show that 40% to 60% of patients have capsular penetration after pathological workup of the prostate. Particularly in terms of PSA-free survival the prognosis closely correlates with pathological stage. Patients with pathologically organ confined cancer achieve PSA-free survival and possible cure rates of greater than 90% for up to 10 years,[7,9,10] whereas capsular penetration (stage pT3a or greater) markedly decreases PSA-free survival and cure rates. An important part of the evaluation of prostate cancer is the analysis of prostatic biopsies for Gleason grade with transrectal ultrasound and digital rectal examination. However, clinical results, such as those of transrectal ultrasonography and digital rectal examination, as well as serum markers, are the first line approach for evaluation.

Biochemical prostate cancer evaluation reveals that neither PSA alone nor in combination (for example percent free PSA, free PSA and so forth) is reliable enough to predict the pathological stage of clinically localized prostate cancer in an individual. A biochemical marker that may add information on pathological staging would enable the clinician to select more carefully patients who have the greatest chance of benefitting from radical prostatectomy.

As a potential new marker, much attention has recently been given to the detection of hK2 in serum and prostatic specimens. For the preoperative evaluation of prostate cancer Catalona et al reported that hK2 combined with percent free PSA may increase the specificity of prostate cancer detection in patients with a total PSA of 2 to 4 ng./ml.[36] Lin et al evaluated hK2 in 52 patients with metastatic prostate cancer.[37] In 7 of the 52 men hK2 was undetectable despite high total PSA. However, hK2 as high as 134 ng./ml. was detected. Becker et al observed that when screening for prostate cancer 65% of patients with a total PSA of less than 3 ng./ml. had detectable hK2 compared to 86% with a total PSA of 3 ng./ml. or greater and a benign biopsy result, and 96% with a PSA of 3 ng./ml. or greater and a biopsy that revealed prostate cancer.[38]

To our knowledge our study is the first to analyze hK2 at different pathological stages and demonstrates the usefulness of hK2 as a potential marker for the biochemical staging of prostate cancer. We verified the ability of serum hK2 to discriminate stage pT2a/b organ confined prostate cancer from stage T3a or greater nonorgan confined disease extension. Serum sampling and rapid processing of samples without previous manipulation of the prostate 1 day preoperatively provided evidence for optimal analytical conditions, that is pathological examination of the prostatectomy specimen according to the Stanford protocol for accurate pathological staging.

An important aspect of our study is that it is based on the analysis of samples measured by 2 different assay generations. Although this technique may be interpreted as a potential weakness, we consider it to be an advantage since it permitted us to eliminate a potential bias due to using a single assay procedure. In each assay generation the results of hK2 detection revealed excellent discrimination for each

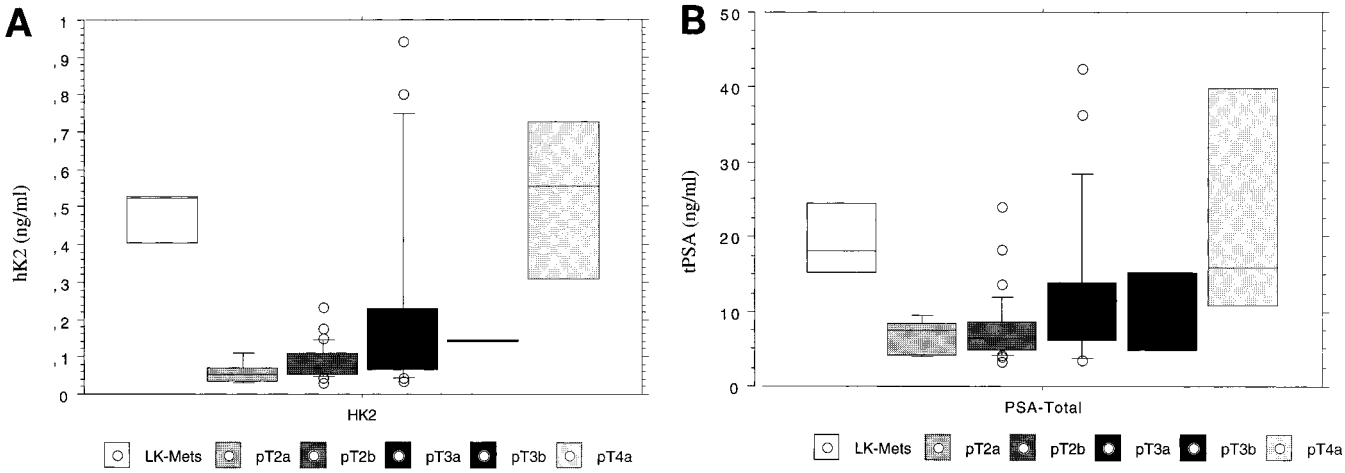

FIG. 2. Box plots of pathological stage. *A*, hK2. *B*, total PSA (*tPSA*)

TABLE 5. *Odds ratios for hK2, and total and free PSA calculated by multivariate logistic regression in organ and nonorgan confined cancers*

	Crude Odds Ratio (95% CI)	Odds Ratio (95% CI)	1 SD
Kallikrein 2	14.5 (1.94–109)	13.3 (0.77–230)	0.20
Free PSA	7.47 (1.74–32.1)	1.45 (0.12–17.9)	1.50
Total PSA	4.35 (1.51–12.6)	0.90 (0.14–5.9)	9.72

TABLE 6. *hK2 in 3 main Gleason groups of organ and nonorgan confined cancer*

Gleason Grade	Mean (ng./ml.)	Median (ng./ml.)	SE	No. Pts.	Min. (ng./ml.)	Max. (ng./ml.)
Organ confined:						
3 + 3 or Less	0.08	0.06	0.01	24	0.03	0.23
3 + 4	0.08	0.07	0.01	13	0.05	0.17
4 + 3 or Greater	0.08	0.08		1	0.08	0.08
Nonorgan confined:						
3 + 3 or Less	0.24	0.11	0.12	6	0.06	0.83
3 + 4	0.17	0.12	0.05	16	0.04	0.71
4 + 3 or Greater	0.54	0.62	0.16	5	0.10	0.94

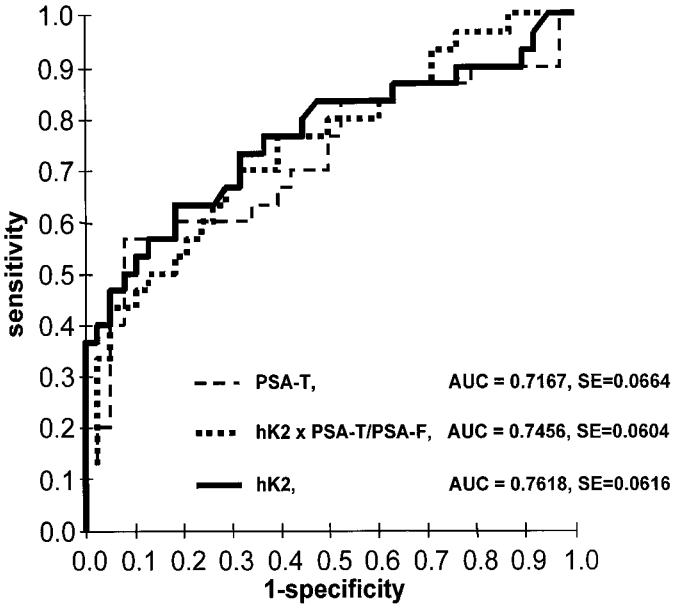

FIG. 3. ROC curves. *PSA-T*, total PSA. *PSA-F*, free PSA. *AUC*, area under curve.

pathological T stage group, which not only shows the consistency of our results, but also permitted us to summarize our data in a single data set and perform subsequent evaluations (table 1).

Table 2 shows that hK2 only and the hK2 × (total PSA/free PSA) algorithm provided the most accurate information for discriminating organ from nonorgan confined cancer ($p < 0.0001$ and $p = 0.005$, respectively), followed by total and free PSA, and nonsignificant percent free PSA. Furthermore, when analyzing each sample cohort by the second and third generation assays for hK2 and PSA, we showed in each set that hK2 was superior to total PSA for discriminating organ from nonorgan confined disease (fig. 1). Because the 75th percentile of the organ confined group was less than the 25th percentile of the nonorgan confined group in set 1, one may argue that the 2 groups were better separated in this than in the second set of patients. We do not believe that this finding is due to a difference in discrimination ability, but rather to different sample sizes and possibly slightly different patient characteristics in the 2 sets. Still despite this difference the discrimination of organ from nonorgan confined disease is superior in each sample set compared with that of total PSA only.

The results of hK2 and total PSA detection according to pathological stage and positive lymph node revealed that hK2 may be detected in the majority of patients (61 of 68) with clinically localized prostate cancer. However, because 7 patients with organ confined cancer had hK2 below the detection limit, it is obvious that there is a need for an assay with an even lower functional detection limit, particularly for evaluating cases that were hK2 negative in our cohort. We identified patients with nonorgan confined cancer but low hK2, and so low hK2 does not guarantee localized carcinoma. On the other hand, elevated hK2, such as 0.20 ng./ml. or greater, was noted in only 1 of 38 patients with stage pT2 cancer. Therefore, a high concentration of hK2 makes extracapsular extension at least likely. The clinical application of hK2 detection in serum is the option of a nerve sparing procedure, which may be performed safely and without compromising the radical nature of the operation when clinical staging and, more importantly, analysis of systematic sextant biopsies indicate organ confined cancer growth. As mentioned previously, single PSA measurements are not specific enough to allow the precise prediction of the final pathological stage.[11,12] This application may also be an example of how hK2 may provide more information than PSA. This suggestion is supported by the fact that no patient in our study with nonorgan confined cancer had undetectable hK2 despite PSA as low as 3.43 ng./ml. in stage pT3a and 4.76 ng./ml. in stage pT3b disease.

Table 4 shows that although pathological stages 2a/b were

distinguished from stages pT3a or greater, the distinction between stages pT2a versus pT2b and pT3a versus pT3b disease did not demonstrate a statistically significant difference. However, the fact that 7 patients had nondetectable hK2 in the stage pT2a/b group strongly limits their evaluation. A more sensitive hK2 assay may provide better analysis but currently we cannot answer this question. A more obvious distinction occurred in pathological stages 3a/b to 4a and lymph node positive disease. However, it would be premature to speculate on that finding due to our small number of patients. We are aware that the inclusion of stage pT4 and lymph node positive cancer may bias the mean and median of nonorgan confined cases (tables 3 and 4). It is important to stress that disease in these patients was also judged to be clinically localized and, thus, they were candidates for radical prostatectomy. Surgery was discontinued in patients with positive lymph nodes. The fact that the perioperative and postoperative evaluation provided information that cancer was far more advanced than clinically expected does not limit the application of hK2 determination for biochemical staging purposes

The calculation of sensitivity and specificity showed that in the most important aspect (prediction of stage pT2a/b disease at 95% sensitivity) hK2 was significantly better than PSA only and the algorithm combining hK2 with PSA. Calculating the odds and crude odds ratios by multivariate logistic regression analysis revealed that only the crude odds ratio indicated a significant advantage for hK2 compared with free and total PSA. The standard odds ratio only demonstrated a nonsignificant advantage for hK2. However, this calculation has limitations because our number of samples was relatively small. In fact, the minimal number of values needed for multivariate logistic regression is 30, which is exactly the number of nonorgan confined cancer cases in our study. Despite these results the crude odds ratio showed that hK2 performed better since even small changes in the hK2 concentrations (increase by 0.2 ng./ml.) strongly elevated the risk of capsular penetration.

Calculation of the mean Gleason score revealed a statistically significant difference in organ versus nonorgan confined cancer (6.1 versus 6.6, $p < 0.01$). Therefore, we correlated more completely Gleason grade with the serum concentration of hK2, which demonstrated that hK2 increased only with advancing cancer dedifferentiation (dominant Gleason grade 4). We divided Gleason grade into grades 3+3 or less, 3+4 and 4+3 or greater. Table 6 shows that the mean, median and range of hK2 were not significantly different in all 3 groups of organ confined cancer. Even the patient with the highest hK2 (0.23 ng./ml.) with organ confined cancer had a Gleason grade of 3+3 or less. One man with stage pT2b cancer and a Gleason score of 4+4 had a hK2 concentration of 0.08 ng./ml. In patients with nonorgan confined cancer the median and range were also comparable. Only in men with a Gleason score of 4+3 or greater were the mean and median Gleason scores 3 and 5-fold higher than in those with Gleason grades 3+4 and 3+3 or less. This finding was also noted by Kwiatkowski et al.[39] By correlating grades 1+2 versus grade 3 disease they identified a 2-fold higher concentration of hK2 in patients with grade 3 disease. However, their grading system cannot be compared with the more meticulous Gleason grading. Moreover, their patients had high mean PSA and no information on clinical or pathological stages was provided.

We believe that serum hK2 more strongly mirrors an increase in pathological stage than does total PSA concentration. The observed increase in hK2 with increasing Gleason score reflects the fact that nonorgan confined cancer had a higher Gleason score than organ confined cancer, a phenomenon that is common in increasing stage disease.

CONCLUSIONS

hK2 only or the algorithm, hK2 × (total PSA/free PSA) improves the preoperative evaluation of patients who undergo radical prostatectomy due to superior separation of organ and nonorgan confined cancer compared with that of total and free PSA, and percent free PSA. Thus, the inclusion of hK2 in the preoperative biochemical evaluation of prostate cancer may provide a substantial improvement in the selection criteria of patients with prostate cancer, particularly with respect to electing nerve sparing radical prostatectomy. Further evaluation of hK2 is necessary. However, our results indicate the potential for hK2 to be a promising tumor marker, perhaps equal to PSA, for adenocarcinoma of the prostate.

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