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Original Article

Serum human glandular kallikrein 2 (hK2) for distinguishing stage and grade of prostate cancer

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Background: Human glandular kallikrein (hK2) has been shown to add important information regarding the early detection and staging of prostate cancer. Preliminary analysis pointed out that hK2 may discriminate between pT2 and pT3 tumors, and that hK2 may predict Gleason grade 4/5 cancer volume, better than prostate-specific antigen (PSA) or percent free PSA (% fPSA). We investigated the role of hK2 serum values for predicting pathological stage, grade and Gleason score.
Methods: Prostate-specific antigen, free PSA and hK2 were measured on 222 untreated prostate cancer patients who had received radical prostatectomy at the Charité Hospital, Berlin, Germany. Pathological work up revealed pT2-cancer in 111 patients and pT3-cancer in 111 patients. Grade 2 was found in 118 patients whereas grade 3 tumors were found in 104 patients.
Results: For pT2 and pT3 patients, the % fPSA ($P = 0.006$), the ratios hK2/fPSA ($P = 0.08$) and hK2 × tPSA/fPSA ($P = 0.002$) were all significant different whereas hK2 ($P = 0.143$) and PSA ($P = 0.1$) did not differ. Between grade 2 and grade 3 tumors, the hK2 alone ($P = 0.27$), the % fPSA ($P = 0.13$), the ratios hK2/fPSA ($P = 0.94$) and hK2 × tPSA/fPSA ($P = 0.12$) did not separate, whereas PSA ($P = 0.039$) showed a difference. The same relationships were found between the two groups in Gleason score <7 and ≥7. Neither the hK2 ratio, nor % fPSA was different.
Conclusion: Human glandular kallikrein was not different between pT2 and pT3, nor between G2 versus G3 or Gleason scores <7 and ≥7 prostate cancer. Together with % fPSA, hK2 may only help to distinguish preoperatively between pT2 and pT3 prostate cancer but cannot add further information.

Key words human glandular kallikrein 2, prostate cancer, prostate specific antigen.

Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in the United States and in other developed countries.[1] Prostate-specific antigen (PSA) is widely used for PCa screening. However, PSA (total PSA, tPSA) lacks specificity and therefore several strategies have been developed to improve the sensitivity and specificity of PCa diagnosis, especially in patients with tPSA levels 2–10 ng/mL, such as PSA density as ratio of tPSA to prostate volume,[2] PSA velocity as PSA change over time[3] or age-specific PSA reference ranges.[4] However, these approaches could only partially fulfill expectations.[5–7] Percentage of free PSA (fPSA) to tPSA (% fPSA) can also improve the accuracy of PCa detection[8,9] and has been established as a US Food and Drug Administration approved marker.[10,11]

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Although some studies could at least partially demonstrate correlations between % fPSA and stage or grade,[12–14] an individual prediction of organ-confined (<T3a) or nonaggressive disease (Gleason score <7) is not possible using % fPSA.[15–18]

Beside PSA (also called human kallikrein 3, hK3), another member of the human kallikrein family,[19] the human glandular kallikrein 2 (hK2) could also have additional value for early detection of PCa.[20–23] The possible physiological function of hK2, which corresponds to about 1–3% of the concentration of tPSA in serum, its molecular forms and relationship to PSA have been extensively reviewed.[24–26] HK2 has the highest homology to PSA with about 80% identity at the amino acid and DNA level.[27] The ratio of hK2 to free PSA or the combination of % fPSA and hK2/fPSA within the tPSA ranges 2–4 and 4–10 ng/mL enhances the discrimination between PCa and BPH patients.[23]

Importantly, hK2 has been reported by Recker *et al*.[28] to discriminate between high (G1 and G2) and low grade (G3) tumors and between Gleason scores 2–6 (<7), 7 and 8–10 (>7).[29] Recently Bangma *et al*.[30] could not substanti-

ate a difference between G1, G2 and G3 PCa if using hK2 or hK2 in combination with tPSA and fPSA.

Regarding a possible discrimination of pathological stage pT2 and pT3 in PCa or the independent prediction of the final stage, Haese and coworkers[16,31–33] found a significant difference in hK2 levels between pT2 and pT3 PCa patients in every study. Again, only the very recent study by Bangma *et al*.[30] could not find a relationship of hK2 to pathological stage. Thus, the aim of our present study on a relatively large number of patients with organ confined and non-organ confined PCa was to investigate:

1 whether hK2 or its ratios with tPSA and fPSA may predict final pathological stage, and
2 whether hK2 or its tPSA and fPSA ratios may differentiate between G1, G2 and G3 PCa, and
3 whether hK2 can distinguish between Gleason score <7 and ≥7.

Patients and methods

This study included a total number of 222 PCa patients (mean age, 62 years; range, 46–75 years) with tPSA concentrations ranging from 0.5 to 48 ng/mL, and with 205 PCa patients (92.3%) had a tPSA between 2 and 20 ng/mL. None of them had received antiandrogen treatment before blood sampling. Serum samples were collected the day before operation and at least 3–4 weeks after prostate manipulation such as prostate biopsy or digital rectal examination (DRE) and stored at −70°C until measurement. Selection criteria was the availability and amount (at least 1 mL) of unthawed serum vials since most the patients were already included in other studies. Total PSA and free PSA were assayed using the Immulite PSA and Immulite Free PSA assays in Berlin, Germany (DPC, Los Angeles, CA, USA). In parallel, hK2 was measured in Toronto, Canada with a recently published research assay.[34]

All patients were treated either with radical laparoscopic, retropubic or perineal prostatectomy at the University Hospital Charité in Berlin, and were diagnosed histopathologically by microscopic examination of the prostatic specimens. Cancer stage was assigned according to the TNM system from 2002, and the histological grade was classified as grade 1, 2 and 3 as previously described in detail.[35] Only patients without metastases were analysed (all N0 and M0).

The pathological stages were the following: pT2a and pT2b ($n = 10$), pT2c ($n = 101$), pT3a ($n = 83$), pT3b ($n = 22$), pT4 ($n = 6$). The patients were subdivided into organ-confined stage (pT2a–pT2c; $n = 111$) and extraprostatic disease (pT3a–pT4; $n = 111$).

The grading were as following: G1a and G1b ($n = 3$), G2a ($n = 37$), G2b ($n = 78$), G3a ($n = 74$), G3b ($n = 30$). Thus, 118 patients were in the group of G1 and G2 tumors and 104 patients in the G3 group.

Gleason grades were available from 197 of the 222 patients and the distribution regarding an increasing Gleason score was as follows: Gleason score 2 ($n = 1$); Gleason score 1 + 2 or 2 + 1 = 3 ($n = 3$); Gleason score 2 + 2 = 4 ($n = 18$); Gleason score 3 + 2 or 2 + 3 = 5 ($n = 31$); Gleason score 4 + 2, 2 + 4 or 3 + 3 = 6 ($n = 28$); Gleason score 2 + 5, 3 + 4, 4 + 3 or 5 + 2 = 7 ($n = 62$); Gleason score 3 + 5, 4 + 4 or 5 + 3 = 8 ($n = 31$); Gleason score 4 + 5 or 5 + 4 = 9 ($n = 21$); Gleason score 10 ($n = 2$).

The patients were subdivided by Gleason score <7 ($n = 81$) and Gleason score ≥7 ($n = 116$) to discriminate nonaggressive and aggressive PCa. A separate comparison of the Gleason score 7 group ($n = 62$) was also performed.

Diagnostic pelvic lymph node dissection was performed in 193 of the 222 PCa patients, but not in the remaining 29 patients. The nodal status of all patients were: pN0, $n = 193$; Nx, $n = 29$. No patient had positive lymph nodes.

Prostate volume (available from 205 of 222 patients) was determined by transrectal ultrasound (TRUS; median 30 cm^3, range 13–140 cm^3) using the prolate ellipse formula $\pi/6 \times$ (transverse diameter × anteroposterior diameter × cephalocaudal diameter).

For statistical analyses the statistical software package, SPSS 11.5 for Windows (SPSS, Chicago, IL, USA) was used. The non-parametric Kruskal–Wallis test of variance and the Mann–Whitney *U*-test were carried out. A two-sided *P*-value lower than 0.05 was considered statistically significant.

Results

The median values of the variables for all patients within the different tPSA ranges are given in Table 1. HK2 alone was never significantly different within all analysed tPSA ranges regarding stage, grade or Gleason score (see also Tables 2–4). PSA only differed within the two largest tPSA ranges between grade G2 and G3 and between Gleason scores <7 and ≥7. However,% fPSA was always significantly different between stages pT2 and pT3 with exception for the tPSA range 2–4 ng/mL with only 33 patients (pT2, $n = 20$; pT3, $n = 13$). The ratio hK2/fPSA was less powerful than the ratio hK2 × tPSA/fPSA to discriminate pT2 and pT3 PCa. Only for the tPSA range 2–4 ng/mL there was no discrimination possible between stages pT2 and pT3 if calculating the ratio hK2 × tPSA/fPSA. Using these hK2 ratios, no significant differentiation between grades G2 and G3 or Gleason scores <7 and ≥7 was achieved. The same results with no differences between grades G2 and G3 or Gleason scores <7 and ≥7 are visible for hK2 and % fPSA. Prostate volume itself was significantly smaller in patients with pT3 compared to pT2 disease within all tPSA ranges except for the 2–4 ng/mL tPSA range.

Table 2 gives the detailed median values for all pT2 and pT3 patients with *P*-values (separated pT2 and pT3 data for all tPSA ranges are analysed but not given). Since hK2 concentrations are lower and % fPSA values are significantly greater in pT2 stages, the ratio of both parameters (hK2 × tPSA/fPSA) revealed the best discrimination between pT2 and pT3 stages with a *P*-value of 0.002.

As above described, hK2 and its ratios could not distinguish between G2 and G3 tumors (Table 3) if analysing all 222 patients. Only tPSA was significantly different between G2 and G3 tumors but not in the tPSA ranges 2–4, 4–10 and 2–10 ng/mL. As expected, significantly more

Table 1 Numbers of patients and median values of hK2, PSA,% fPSA, hK2/fPSA, hK2 × tPSA/fPSA, prostate volume and age within all investigated tPSA ranges

tPSA range (ng/mL)	Number of patients (with available Gleason)	HK2 (ng/mL)	tPSA (ng/mL)	% fPSA	HK2/ fPSA	hK2 × PSA/ fPSA	Prostate volume	Age (years)
0.5–48	222 (197)	0.221	7.3‡§	8.83†	0.352	2.55†	30 ccm†	63
2–20	205 (182)	0.221	7.3‡§	8.59†	0.367	2.61†	30 ccm†	63‡
2–10	143 (126)	0.201	5.9	9.13†	0.396†	2.28†	28 ccm†	63†‡
2–4	33 (29)	0.154	3.0	13.2§	0.417	1.25	27.5 ccm	64
4–10	110 (97)	0.218	6.7	8.35†	0.388†	2.63†	29 ccm†	64†‡

†significantly different between stages pT2 and pT3 ($P < 0.05$); ‡significantly different between grades G2 and G3 ($P < 0.05$); §significantly different between Gleason scores <7 and ≥7 ($P < 0.05$). tPSA, Total PSA; hK2, human glandular kallikrein 2; % fPSA, percent free PSA.

Table 2 Comparison of stage pT2 and pT3 PCa with medians of hK2, PSA,% fPSA, hK2/fPSA, hK2 × tPSA/fPSA, prostate volume, age, grade and Gleason score

	HK2 ng/mL	PSA ng/mL	% fPSA	HK2/ fPSA	hK2 × tPSA/fPSA	Prostate volume	Age	Grade	Gleason score
Stage pT2	0.204	7.0	9.4	0.323	2.14	32 ccm	62	2	<7
Stage pT3	0.226	8.0	8.1	0.386	2.96	28 ccm	63	3	≥7
P-value	0.14	0.11	0.006*	0.08	0.002*	0.009*	0.27	<0.001	<0.001

*Significantly different ($P < 0.01$). tPSA, Total PSA; hK2, human glandular kallikrein 2; % fPSA, percent free PSA.

Table 3 Comparison of grade G2 and G3 PCa with medians of hK2, PSA,% fPSA, hK2/fPSA, hK2 *tPSA/fPSA, prostate volume, age, stage and Gleason score

	HK2 ng/mL	PSA ng/mL	% fPSA	HK2/ fPSA	hK2 × tPSA/fPSA	Prostate volume	Age	Stage	Gleason score
Grade G2	0.214	6.9	9.0	0.346	2.44	30 ccm	61	2	<7
Grade G3	0.230	8.3	8.6	0.361	2.73	29 ccm	63.5	3	≥7
P-value	0.27	0.03*	0.13	0.94	0.12	0.72	0.14	<0.001	<0.001

*Significantly different ($P < 0.05$). tPSA, Total PSA; hK2, human glandular kallikrein 2; % fPSA, percent free PSA.

pT2 and Gleason score <7 tumors are found in the group with G2 prostate cancers.

The differentiation of the 197 patients with available Gleason scores and the subdivision in Gleason scores <7 and ≥7 is shown in Table 4. There is a similar behavior visible as for the G2 and G3 tumors. Only tPSA was significantly different between Gleason scores <7 and ≥7 whereas hK2,% fPSA, hK2/fPSA, hK2 × tPSA/fPSA, prostate volume and age showed no difference.

The separate comparison of the Gleason score 7 group ($n = 62$) with the patient group with Gleason scores <7 ($n = 81$) and Gleason scores >7 ($n = 54$) also showed no difference for hK2,% fPSA, hK2/fPSA, hK2 × tPSA/fPSA, prostate volume and age with exception for tPSA ($P = 0.025$; Kruskal–Wallis). There was no difference for all parameters if comparing the Gleason scores <7 group with the Gleason score 7 group and the Gleason score 7 group with the Gleason scores >7 group. Only the comparison of the Gleason scores <7 with the Gleason scores >7 gave a significant difference for tPSA ($P = 0.008$) and % fPSA ($P = 0.03$) but not for the other parameters.

To summarize, hK2 could not distinguish between stages T2 and T3 as well as grades G2 and G3 nor Gleason scores <7 and ≥7. Only % fPSA and the ratio hK2 × tPSA/fPSA were able to discriminate between stages pT2 and T3 in PCa patients. However, at the low PSA range 2–4 ng/mL % fPSA could significantly distinguish between Gleason scores <7 and ≥7 but also not between G2 and G3 PCa. TPSA was significantly higher in more aggressive PCa as G3 and Gleason scores ≥7.

Table 4 Comparison of Gleason score <7 and ≥7 PCa with medians of hK2, PSA,% fPSA, hK2/fPSA, hK2 *tPSA/fPSA, prostate volume, age, stage and grade

	HK2 ng/mL	PSA ng/mL	% fPSA	HK2/ fPSA	hK2 × tPSA/fPSA	Prostate volume	Age	Stage	Grade
Gleason score <7	0.210	6.8	9.6	0.351	2.32	29 ccm	61	2	2
Gleason score ≥7	0.227	8.3	8.6	0.350	2.72	30 ccm	63	3	3
P-value	0.33	0.04*	0.1	0.83	0.12	0.46	0.33	<0.001	<0.001

*Significantly different ($P < 0.05$). tPSA, Total PSA; hK2, human glandular kallikrein 2; % fPSA, percent free PSA.

Discussion

Within the last years many studies have proven the additional value of hK2 to enhance the discrimination between PCa and BPH.[20–23] Early immunohistological studies on hK2 showed an increased staining from benign tissue over prostate intraepithelial neoplasia, prostate cancer to lymph node metastasis.[36,37] These findings in prostate tissue supported the hypothesis of higher hK2 serum concentrations in pT3 versus pT2 and more aggressive PCa (G3 vs G2, Gleason score <7 vs ≥7) as found in many studies.[16,28,29,31–33]

Recently, Magklara *et al*.[38] measured hK2 and PSA quantitatively in cancerous and non-cancerous prostatic tissue. Both prostate kallikreins were expressed more in non-cancerous than in cancerous prostatic tissue demonstrating that both PSA and hK2 are down-regulated in prostate cancer compared with non-cancerous tissue.[38] However, the degree of down-regulation was higher for PSA than for hK2.[38] Thus, the relative amount of the hK2 protein is larger in cancerous than in benign prostate tissue. Using quantitative reverse transcription–polymerase chain reaction, a very recent study confirmed that the ratio of relative expression of hK2 to PSA mRNA is higher in cancerous G2 and G3 tissue compared to normal prostate tissue.[39] Despite a higher relative amount of hK2 at the RNA and protein level in PCa tissue compared to benign prostate tissue,[38,39] these quantitative measurements could not confirm the expected absolute higher amounts of hK2 in PCa tissue compared to benign prostate tissue as primarily found by immunohistological studies.[36,37] Thus, the decreased tissue expression of the hK2 gene and hK2 protein in PCa is unlikely to be responsible for higher serum concentration in advanced stage and high grade PCa as earlier described by others.[28,29,31]

Our measured hK2 concentrations are in concordance with very recent data obtained by Bangma *et al*.[30] The authors showed in 142 men with BPH, and 146 with PCa that pro PSA, a precursor forms of PSA and hK2, alone or combined, did not improve the specificity of fPSA for discriminating BPH and PCa.[30] Furthermore, there was no correlation between these serum markers and pathological tumor grade. They concluded that the clinical effect of using pro PSA or hK2 for detecting and grading prostate cancer remains limited.[30] We also found no discriminating power for hK2 to distinguish pT2 and pT3, G2 and G3 or Gleason scores <7 and ≥7. This is in contrast to data obtained by Haese *et al*.[31–33] A smaller number of patients especially for pT3 stages in the early studies by Haese *et al*.[31,32] may only partially explain the differences. In another study by Haese *et al*.[33] with the largest number of patients, the behavior of hK2 was similar to their previous studies. Another explanation might be the use of different hK2 assays. So far, no commercially available hK2 assays exist and there is not universally accepted calibrator for hK2 like there is for PSA. There have been three hK2 assays available to date: (i) the assay in the present study;[34] (ii) the Beckman Coulter total hK2 assay;[40] and (iii) the Turku hK2 assay.[41] The hK2 assay used in this study measured about 1.5-fold higher concentrations when compared with the Beckman Coulter hK2 research assay (obtained on 29 samples, data not shown). Two comparisons of the Beckman Coulter hK2-assay and the Turku hK2 assay were recently performed by Haese *et al*.[42] and Blijenberg *et al*.[43] Differences were found between these two assays that can not be attributed to calibration differences. Whereas Recker *et al*.[28] found significant differences by using another hK2 assay compared to the present study, Nam *et al*.[29] used the same assay as our study and they could also substantiate a significant difference between Gleason scores <7, 7 and >7. Data from this study could not confirm these significant differences between aggressive and non-aggressive PCa. Only between Gleason scores <7 and >7 there was a difference between PSA and % fPSA but not between hK2 levels. It is mentionable that in this study with only PCa patients the amount of hK2 to tPSA which is usually 1–3% was slightly higher with 3.0–5.1% within the different tPSA ranges.

The ratios hK2/fPSA and hK2 × tPSA/fPSA were the only hK2 parameters with significant differences between pT2 and pT3 PCa. This effect is probably mostly caused by the significance of % fPSA between pT2 and pT3 since hK2 alone was not different. Other studies also found an inverse relationship of % fPSA to the pathological stage.[44–46] A large prospective study on 379 men with tPSA concentrations between 4 and 10 ng/mL revealed % fPSA to be the strongest predictor of postoperative pathological outcome.[14] However, the use of % fPSA is controversial since others noted no significant improvement in staging using % fPSA.[15–17] Very recently, Miyake *et al*.[18] could also not prove an advantage in using % fPSA to distinguish pT2 and pT3 PCa in a Japanese population. Thus, both hK2 and % fPSA show contrary results for staging and grading PCa.

Conclusion

To conclude, this study could neither find a difference in hK2 level between pT2 and pT3 PCa nor between G2 versus G3 PCa or Gleason scores <7 and ≥7 PCa. There is a need for better pathological and serological biomarkers to improve PCa detection and differentiation between organ-confined versus local advanced PCa, and more importantly to improve differentiation between low grade and more aggressive PCa.

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