Published in final edited form as:

Prostate. 2020 January ; 80(1): 51–56. doi:10.1002/pros.23916.

Kallikrein markers performance in pretreatment blood to predict early prostate cancer recurrence and metastasis after radical prostatectomy among very high-risk men

Melissa J. Assel, MS^a, Hans David Ulmert, MD, PhD^{b,d,g}, R. Jeffery Karnes, MDⁱ, Stephen A. Boorjian, MD^j, David W. Hillman, MS^j, Andrew J. Vickers, PhD^a, George G. Klee, MD, PhD^h, Hans Lilja, MD, PhD^{c,e,g,*}

^aDepartment of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

^bMolecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

^cDepartments of Laboratory Medicine, Surgery, and Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

^dDepartment of Molecular and Medical Pharmacology, David Geffen School of Medicine at University of California, Los Angeles, CA, USA

^eNuffield Department of Surgical Sciences, University of Oxford, Oxford, UK

^fDivision of Oncology, Clinical Sciences, Lund University, Malmö, Sweden

^gDepartment of Translational Medicine, Lund University, Malmö, Sweden

^hMayo Clinic College of Medicine & Science, Mayo Clinic, Rochester, MN, USA

ⁱDepartment of Urology, Mayo Clinic, Rochester, MN, USA

^jBiomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA

Abstract

Background: To assess whether a prespecified statistical model based on the four kallikrein markers measured in blood—total, free, and intact prostate-specific antigen (PSA), together with human kallikrein-related peptidase 2 (hK2)—or any individual marker measured in pretreatment

*Corresponding author: Hans Lilja, MD, PhD, 1275 York Ave, BOX 213, New York, NY 10065, (P) 212-639-6982, (F) 646-422-2379, liljah@mskcc.org.

Disclosure / Conflict of Interest Statement

The work was supported in part by grants from the National Cancer Institute [R01 CA160816, R01 CA175491, P50 CA092629, and P30 CA008748]; the Sidney Kimmel Center for Prostate and Urologic Cancers; David H. Koch through the Prostate Cancer Foundation; the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre Program in the United Kingdom; the Swedish Cancer Society (CAN 2017/559); and the Swedish Research Council (VR-MH project no. 2016-02974).

Hans Lilja holds patents for intact PSA assays, and is named, along with Andrew J. Vickers, on a patent for a statistical method to detect prostate cancer. The patent application for the statistical model have been licensed and commercialized as the 4KscoreTM by OPKO Diagnostics. Drs. Vickers and Lilja receive royalties from sales of this test. Additionally, Dr. Lilja owns stock and Dr. Vickers owns stock options in OPKO. George G. Klee is the co-inventor of hK2-related patents held by Mayo Foundation for Medical Education and Research: “Antibodies specific for human prostate glandular kallikrein” (USA) and “Methods to detect hK2 polypeptides” (Europe).

serum were associated with biochemical recurrence-free (BCR) or metastasis-free survival after radical prostatectomy (RP) in a subgroup of men with very high-risk disease.

Methods: We identified 106 men treated at Mayo Clinic from 2004 to 2008 with pathological Gleason Grade Group 4–5 or seminal vesicle invasion at radical prostatectomy. Univariable and multivariable Cox models were used to test the association between standard predictors (Kattan nomogram and GPSM [Gleason, PSA, seminal vesicle and margin status] score), kallikrein panel, and individual kallikrein markers with the outcomes.

Results: BCR and metastasis occurred in 67 and 30 patients, respectively. The median follow-up for patients who did not develop a BCR was 10.3 yr (IQR: 8.2–11.8). In this high-risk group, neither Kattan risk, GPSM score, or the kallikrein panel model was associated with either outcome. However, after adjusting for Kattan risk and GPSM score, separately, preoperative intact PSA was associated with both outcomes while hK2 was associated with metastasis-free survival.

Conclusions: Conventional risk prediction tools were poor discriminators for risk of adverse outcomes after RP (Kattan risk and GPSM risk) in patients with very high-risk disease. Further studies are needed to define the role of individual kallikrein marker forms in blood to predict adverse prostate cancer outcomes after RP in this high-risk setting.

Keywords

Kallikreins; Prediction models; Prostate cancer; Prostate-specific antigen

Introduction

Prostate cancer screening with prostate-specific antigen (PSA) has been widely recommended as an indication for biopsy.^{1–3} Measured PSA is a series of different molecular isoforms: complexed PSA vs free PSA, intact vs nicked PSA, and human kallikrein-related peptidase 2.^{4–6} Measuring isoforms separately has been shown to improve prediction of biopsy results among men with elevated PSA.^{7,8} Four kallikrein markers in blood—total prostate specific antigen (tPSA), free prostate specific antigen (fPSA), intact prostate specific antigen (iPSA), and kallikrein-related peptidase 2 (hK2)—are included in the kallikrein panel which is now commercially available as the 4Kscore™ which has been subject to 12 clinical validation studies ($n = 11134$ biopsies), with a pooled discrimination of 0.81 in predicting risk of Gleason score 7 or higher risk of prostate cancer.⁹ The kallikrein markers were combined using a previously developed model to predict risk of Gleason score 7 or higher (high-grade) prostate cancer based on 6129 biopsied men in the UK Prostate Testing for Cancer and Treatment (ProtecT) study¹⁰, the standard algorithm that is used for the 4Kscore in clinical practice. Among men with elevated PSA, the prespecified kallikrein marker panel risk score has been shown to be highly predictive of the long-term risk of metastasis and death in two large, independent cohorts of men not subject to PSA screening aged 50–60 and 45–73 years, respectively.^{11,12} The addition of free PSA and hK2 has been shown to improve discrimination of biochemical recurrence (BCR) after radical prostatectomy (RP) both to a prediction model based on total PSA alone and to a prediction model based on clinical stage, biopsy Gleason score and total PSA.^{13–15}

Our aim was to determine whether the prespecified kallikrein marker panel risk or any individual kallikrein marker form in pretreatment samples is associated with BCR after RP among men with very high-risk disease, defined as Gleason Grade Group 4–5 or seminal vesicle invasion at RP. Furthermore, we aimed to determine whether the kallikrein panel or individual markers could improve predictions of BCR or development of metastasis based on established predictive models: the Kattan preoperative nomogram and the Gleason, PSA, seminal vesicle and margin status (GPSM) risk score for postoperative features.

Materials and Methods

After receiving approval from the Mayo Clinic IRB, we identified 106 men who underwent open or robotic RP for prostate cancer at the Mayo Clinic from 2004 to 2008 and were considered very high-risk for recurrence who participated in the Mayo Prostate SPORE study. None of these 106 men received neo-adjuvant hormones prior to RP. High risk was defined as a pathological Gleason Grade Group 4–5 or SVI at RP. Biomarker levels for the kallikrein panel were measured in plasma samples collected prior to RP. BCR was defined as a PSA ≥ 0.4 ng/ml at least 30 days after RP. Metastases were determined via bone scans.

Total and free PSA levels were measured using the AutoDelfia 1235 automatic immunoassay system using the dual-label DELFIA ProstatuS total/free PSA-Assay (Perkin-Elmer, Turku, Finland) calibrated against the World Health Organization (WHO) 96/670 (PSA-WHO) and WHO 68/668 (free PSA-WHO) standards. Intact PSA and hK2 were measured with F(ab')₂ fragments of the monoclonal capture antibodies to reduce the frequency of nonspecific assay interference, as described in detail previously.^{12,16} Ten mL tubes of EDTA blood were collected from consenting subjects by venipuncture. The tubes were spun at 2800 RPM for 10 minutes at 4 degrees Celsius. The plasma was divided in 1 mL aliquots placed in 2 mL vials with screw-top caps with O-rings. The vials were frozen at -80 degrees Celsius. One aliquot from each subject was shipped from Mayo Clinic in Rochester Minnesota to Malmö on dry ice, where they were stored at -80 degrees Celsius until the markers were measured.

Our hypothesis was that the kallikrein panel or the individual components; tPSA, free-to-total PSA ratio, iPSA or hK2 in pretreatment samples, could enhance the discrimination of BCR or metastasis compared to the discrimination of the preoperative or postoperative risk score. First, we tested whether tPSA, free-to-total PSA ratio, iPSA, or hK2 and the previously developed kallikrein model (ProtecT)¹⁰ was associated with BCR using univariable Cox proportional hazards regression. We allowed for cubic splines for tPSA and free-to-total PSA ratio. Next, we assessed whether the significantly associated kallikrein markers or panel aided in the prediction of BCR after adjusting for preoperative (Kattan nomogram) and postoperative (GPSM) scores, separately. The Kattan nomogram uses pre-operative total prostate-specific antigen (PSA), clinical stage, and Gleason grade at biopsy to predict BCR after RP, where BCR is defined as a postoperative PSA ≥ 0.4 ng/ml.¹⁷ GPSM is a scoring algorithm that was developed to predict recurrence after RP using Gleason grade, pre-operative total PSA, seminal vesicle invasion (SVI) and surgical margin status.¹⁸ We evaluated the increment in predictive accuracy with the additional kallikrein markers or panel to the base models that included preoperative and postoperative score using Harrell's

C-index. Bootstrap methods were used to correct for optimism in the multivariable models and generate the 95% confidence intervals. We repeated this analysis for the outcome of metastases-free survival; however, due to the small number of metastasis events we did not include cubic splines for total PSA and free-to-total PSA ratio. As it would be unusual for a urologist to perform additional workup or treatment for those with a total PSA > 50 ng/mL all analyses were performed excluding those with a total PSA > 50 ng/mL and repeated excluding those with a total PSA > 25 ng/mL as sensitivity analyses. All statistical analyses were conducted using Stata 15.0 (Stata Corporation, College Station, TX).

Results

Our initial analysis found no significant association between BCR and either the Kattan nomogram (preoperative risk score) or GPSM score (postoperative risk score) (p-values = 0.2 and 0.2, respectively). Given this surprising result, we examined the data further and identified three patients with very high PSAs, consistent with metastatic disease (PSA of 73 ng/mL, 87 ng/mL, and 198 ng/mL). The clinical and pathological characteristics after exclusion of these patients are given in Table 1. 90% of patients had a Gleason Grade Group of 2 or higher on biopsy, 70% of patients had Gleason Grade Group 4–5 on surgical pathology, and 54% had seminal vesicle invasion. Median of the Kattan nomogram in predicting 5-year risk of BCR after radical prostatectomy was 33% (interquartile range [IQR]:20–50%) and the median GPSM score was 11 (IQR: 10–12), confirming that a cohort at high risk (14%; IQR 7–28) for having a clinically significant tumor on biopsy according to the prespecified four kallikrein marker panel model is representative of our sample set which includes patients at a high risk of recurrence. BCR and metastasis occurred in 67 and 30 patients, respectively. The median follow-up for patients who did not develop a BCR was 10.3 yr (IQR: 8.2–11.8).

Levels of intact PSA, and hK2 were significantly associated with the risk of BCR and metastasis (Table 2). The relationship between BCR and free-to-total PSA was significant, although the relationship between free-to-total PSA ratio and metastasis is in the opposite direction to that expected with a higher ratio associated with greater risk. We found that one observation with the largest free-to-total PSA ratio of 0.5 (nearly 50% greater than the next highest value) was driving the relationship. After we excluded the outlier of free-to-total PSA ratio of 0.5, free-to-total PSA was no longer significantly associated with BCR (p=0.4) nor was it significantly associated with BCR after adjusting for preoperative and postoperative risk scores (p=0.4 and 0.4, respectively). The association between levels of intact PSA and the outcomes remained and the association between levels of hK2 and metastasis progression were statistically significant after adjusting for the Kattan nomogram and the GPSM score (Table 3). The association between hK2 and BCR did not meet conventional levels of significance after adjusting for the Kattan nomogram and GPSM score however, the hazard ratio estimates were similar to those found for the association between hK2 and metastasis progression (Table 3).

For the outcome of BCR, discrimination was poor for the Kattan nomogram (0.596; 95% CI: 0.525, 0.668) and GPSM score (0.576; 95% CI: 0.488, 0.646) (Table 4). Given that the upper bound of the 95% CIs excludes adequate discrimination, we conclude that these standard

prediction models have poor performance in this high-risk setting. Also, the prespecified four kallikrein marker panel risk score demonstrated poor discrimination. There was no meaningful increase in discrimination with the addition of hK2 except when predicting metastasis progression when added to preoperative risk (0.058 increase; Table 4). The addition of intact PSA resulted in meaningful increases in discrimination for all outcome and model combinations with the smallest increase being 0.011 (Table 4).

Results were similar corresponding to the sensitivity analyses excluding men with a PSA greater than 25 ng/mL except that on univariable analysis we did not have sufficient evidence of an association between hK2 and BCR ($p=0.11$; Supplementary Table 1) and that on multivariable analysis after adjusting for preoperative risk the association between intact PSA and BCR did not meet conventional levels of significance ($p=0.072$, Supplementary Table 2). However, for the outcome of metastasis progression the association with intact PSA and hK2 remained significant after adjusting for preoperative and postoperative risk (all p -values <0.011 ; Supplementary Table 2).

There smallest increase in discrimination with intact PSA was 0.009 while the largest increase with hK2 was 0.044 (Supplementary Table 3).

Discussion

The conventional risk prediction tools were poor discriminators for risk of adverse outcomes after RP (Kattan risk and GPSM) in patients with very high-risk disease. Additionally, the prespecified kallikrein panel score demonstrated poor discrimination. However, we demonstrated promising results for certain individual kallikrein forms in pretreatment blood. We found evidence to suggest that intact PSA aided in the prediction of biochemical recurrence on top of Kattan risk score or GPSM and there was promising evidence for the association of hK2.

The Kattan risk score and GPSM are standard risk prediction tools and have been established as useful discriminators of adverse events in the postoperative setting^{18,19} however, they have not been previously explored in a homogeneous group of very high-risk patients. Similarly, there is evidence that the kallikrein panel (4Kscore), and other marker panels such as the Prostate Health Index (PHI) which includes the PSA isoform [–2]proPSA in addition to tPSA and fPSA²⁰, are predictive of adverse events, but again, they have not been explored in very high-risk patients.

In this very high- risk setting we did not find that total PSA aided in predicting BCR or metastasis. This differs from the report from Carlsson *et al.* which established the predictive value of total PSA in predicting BCR in patients with established lymph node positive disease on RP.²¹ That we were not able to establish this relationship might be explained by our smaller sample size (261 versus 106) and that the men in our study had higher risk disease including higher rates of SVI (54% versus 43%) and Gleason 8 disease (69% vs 52%) by our cohort definition.

Although hK2 (*KLK2*) and PSA (*KLK3*) are both well-characterized downstream androgen receptor pathway biomarkers, with 80% amino acid homology²², recent findings have

pointed out important biological differences in relation to resistance to treatment. For instance, in enzalutamide-resistant disease with glucocorticoid receptor cross-activated androgen receptor pathway, *KLK2* expression, but not *KLK3*, is restored²³. This suggests that hK2 is a more relevant biomarker of aggressive disease. The mechanism for the association between intact PSA and outcome in these patients requires further elucidation.

The major limitation of our study is the small number of events. Our findings suggest we are underpowered to identify factors associated with metastasis after RP—likely driven by the limited number of biochemical recurrences and metastatic events observed—and we cannot, therefore, exclude possible associations that we did not find to be statistically significant. Further evaluation of the kallikrein panel and its individual markers is needed in a larger dataset to establish the role they may play in predicting oncologic outcomes after radical prostatectomy.

Conclusions

Standard methods to discriminate risk of adverse outcomes after RP (Kattan risk and GPSM risk) are of limited value in men with very high-risk disease and the kallikrein panel model was also a poor discriminator. However, our results show that studies of individual kallikrein marker measurements are warranted to further assess the prognostic value of hK2 and intact PSA levels in preoperative blood from this subgroup of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Mottet N, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *European urology*. 2017;71(4): 618–629. [PubMed: 27568654]
2. Grossman DC, Curry SJ, Owens DK, et al. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *Jama*. 2018;319(18):1901–1913. [PubMed: 29801017]
3. Carroll PR, Parsons JK, Andriole G, et al. NCCN Guidelines Insights: Prostate Cancer Early Detection, Version 2.2016. *J Natl Compr Canc Netw*. 2016;14(5):509–519. [PubMed: 27160230]
4. Lilja H, Christensson A, Dahlen U, et al. Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clinical chemistry*. 1991;37(9):1618–1625. [PubMed: 1716536]
5. Nurmikko P, Pettersson K, Piironen T, Hugosson J, Lilja H. Discrimination of prostate cancer from benign disease by plasma measurement of intact, free prostate-specific antigen lacking an internal cleavage site at Lys145-Lys146. *Clinical chemistry*. 2001;47(8):1415–1423. [PubMed: 11468231]
6. Piironen T, Lovgren J, Karp M, et al. Immunofluorometric assay for sensitive and specific measurement of human prostatic glandular kallikrein (hK2) in serum. *Clinical chemistry*. 1996;42(7):1034–1041. [PubMed: 8674186]
7. Christensson A, Bjork T, Nilsson O, et al. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. *The Journal of urology*. 1993;150(1):100–105. [PubMed: 7685416]
8. Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific

- antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. *Cancer research*. 1991;51(1):222–226. [PubMed: 1703033]
9. Zappala SM, Scardino PT, Okrongly D, Linder V, Dong Y. Clinical performance of the 4Kscore Test to predict high-grade prostate cancer at biopsy: A meta-analysis of us and European clinical validation study results. *Reviews in urology*. 2017;19(3):149–155. [PubMed: 29302237]
 10. Bryant RJ, Sjöberg DD, Vickers AJ, et al. Predicting high-grade cancer at ten-core prostate biopsy using four kallikrein markers measured in blood in the ProtecT study. *Journal of the National Cancer Institute*. 2015;107(7).
 11. Sjöberg DD, Vickers AJ, Assel M, et al. Twenty-year Risk of Prostate Cancer Death by Midlife Prostate-specific Antigen and a Panel of Four Kallikrein Markers in a Large Population-based Cohort of Healthy Men. *European urology*. 2018;73(6):941–948. [PubMed: 29519548]
 12. Stattin P, Vickers AJ, Sjöberg DD, et al. Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study. *European urology*. 2015;68(2):207–213. [PubMed: 25682340]
 13. Steuber T, Vickers AJ, Serio AM, et al. Comparison of free and total forms of serum human kallikrein 2 and prostate-specific antigen for prediction of locally advanced and recurrent prostate cancer. *Clinical chemistry*. 2007;53(2):233–240. [PubMed: 17185368]
 14. Wenske S, Korets R, Cronin AM, et al. Evaluation of molecular forms of prostate-specific antigen and human kallikrein 2 in predicting biochemical failure after radical prostatectomy. *International journal of cancer*. 2009;124(3):659–663. [PubMed: 19003994]
 15. Steuber T, Vickers AJ, Haese A, et al. Risk assessment for biochemical recurrence prior to radical prostatectomy: significant enhancement contributed by human glandular kallikrein 2 (hK2) and free prostate specific antigen (PSA) in men with moderate PSA-elevation in serum. *International journal of cancer*. 2006;118(5):1234–1240. [PubMed: 16152616]
 16. Vaisanen V, Peltola MT, Lilja H, Nurmi M, Pettersson K. Intact free prostate-specific antigen and free and total human glandular kallikrein 2. Elimination of assay interference by enzymatic digestion of antibodies to F(ab')₂ fragments. *Analytical chemistry*. 2006;78(22):7809–7815. [PubMed: 17105175]
 17. Kattan MW, Eastham JA, Stapleton AM, Wheeler TM, Scardino PT. A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *Journal of the National Cancer Institute*. 1998;90(10):766–771. [PubMed: 9605647]
 18. Thompson RH, Blute ML, Slezak JM, Bergstralh EJ, Leibovich BC. Is the GPSM Scoring Algorithm for Patients With Prostate Cancer Valid in the Contemporary Era? *The Journal of urology*. 2007;178(2):459–463. [PubMed: 17561132]
 19. Kattan MW, Wheeler TM, Scardino PT. Postoperative nomogram for disease recurrence after radical prostatectomy for prostate cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1999;17(5):1499–1507. [PubMed: 10334537]
 20. Hori S, Blanchet JS, McLoughlin J. From prostate-specific antigen (PSA) to precursor PSA (proPSA) isoforms: a review of the emerging role of proPSAs in the detection and management of early prostate cancer. *BJU international*. 2013;112(6):717–728. [PubMed: 22759214]
 21. Carlsson SV, Tafe LJ, Chade DC, et al. Pathological features of lymph node metastasis for predicting biochemical recurrence after radical prostatectomy for prostate cancer. *The Journal of urology*. 2013;189(4):1314–1318. [PubMed: 23085057]
 22. Schedlich LJ, Bennetts BH, Morris BJ. Primary structure of a human glandular kallikrein gene. *DNA (Mary Ann Liebert, Inc)*. 1987;6(5):429–437.
 23. Arora VK, Schenkein E, Murali R, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell*. 2013;155(6):1309–1322. [PubMed: 24315100]

Table 1 -

Patient characteristics for patients with total PSA < 50 ng/mL, continuous estimates are reported as median (Quartile 1, Quartile 3) and categorical estimates as number (percentage).

Characteristic	N=103
Age at Surgery	64 (58, 68)
Gleason Grade Group at Biopsy	
1	10 (10%)
2–3	43 (42%)
4–5	50 (49%)
Gleason Grade Group at Pathology	
1	3 (2.9%)
2–3	28 (27%)
4–5	72 (70%)
Clinical T Stage	
T1C	33 (32%)
T2A	32 (31%)
T2B	15 (15%)
T2C	17 (17%)
T3	5 (4.9%)
Unknown	1 (1.0%)
Total PSA (ng/mL)	8.2 (5.3, 12.6)
Free PSA (ng/mL)	1.2 (0.9, 2.0)
Intact PSA (ng/mL)	0.3 (0.2, 0.6)
HK2 (ng/mL)	0.043 (0.026, 0.081)
Positive Surgical Margin	26 (25%)
Lymph Node Invasion	22 (21%)
Seminal Vesical Invasion	54 (52%)
Kallikrein Predictive Model (%)	14 (7, 28)
Preoperative Risk Score (%)	33 (20, 50)
Postoperative Risk Score	11 (10, 12)

Table 2 -

Univariable Cox regression models for the outcomes of biochemical recurrence and metastases for patients with total PSA < 50 ng/ml (n=103).

	Biochemical recurrence			Metastasis progression		
	HR	95% CI	p value	HR	95% CI	p value
Preoperative risk score (%)	3.59	1.09, 11.85	0.036	5.80	0.96, 35.03	0.055
Postoperative risk score	1.11	0.98, 1.26	0.11	1.38	1.14, 1.66	0.001
Kallikrein predictive model (%)	1.95	0.62, 6.08	0.3	2.84	0.61, 13.18	0.2
Total PSA (ng/ml) *	NA	NA	0.4	1.04	1.00, 1.08	0.065
Free-to-total PSA ratio (increase per 0.01) * [†]	NA	NA	0.036	1.03	0.98, 1.09	0.2
Intact PSA (ng/ml)	2.85	1.58, 5.14	0.001	2.84	1.62, 4.98	0.0003
hK2 (increase per 0.1 ng/ml presented)	1.48	1.08, 2.01	0.013	1.85	1.36, 2.52	<0.0001

HR = hazard ratio; CI = confidence interval; PSA = prostate-specific antigen; hK2 = human kallikrein-related peptidase 2

* HR and CIs not reported as non-linear terms (cubic splines) used for the outcome of BCR.

[†] Significance for BCR highly influenced by a free-to-total PSA outlier.

Table 3 –

Multivariable Cox regression models for the outcomes of biochemical recurrence and metastases for patients with total PSA < 50 ng/ml (n=103).

Marker	Covariate	Biochemical recurrence			Metastasis progression		
		HR	95% CI	p value	HR	95% CI	p value
Intact PSA (ng/ml)	Preoperative risk score (%)	2.52	1.27, 4.99	0.008	2.62	1.42, 4.82	0.002
	Postoperative risk score	2.72	1.41, 5.27	0.003	2.30	1.21, 4.36	0.011
hK2 (increase per 0.1 ng/ml presented)	Preoperative risk score (%)	1.33	0.94, 1.90	0.11	1.75	1.25, 2.45	0.001
	Postoperative risk score	1.40	0.97, 2.00	0.070	1.60	1.12, 2.30	0.011

Discrimination (Harrell's C-index) of various models predicting biochemical recurrence and metastasis progression for patients with total PSA <50 ng/ml. Bootstrap methods were used to correct for optimism and generate the 95% confidence intervals.

Table 4 –

Outcome	Model	Univariable	Adjusted for iPSA	Adjusted for hK2	Difference adding iPSA	Difference adding hK2
Biochemical Recurrence	Preoperative risk score (%)	0.596 (0.525, 0.668)	0.611 (0.531, 0.683)	0.597 (0.512, 0.677)	0.015 (−0.026, 0.076)	0.001 (−0.034, 0.047)
	Postoperative risk score	0.576 (0.488, 0.646)	0.607 (0.515, 0.683)	0.570 (0.475, 0.658)	0.032 (−0.026, 0.106)	−0.005 (−0.071, 0.061)
Metastasis Progression	Preoperative risk score (%)	0.600 (0.508, 0.693)	0.658 (0.550, 0.757)	0.658 (0.542, 0.761)	0.057 (−0.012, 0.158)	0.058 (−0.016, 0.146)
	Postoperative risk score	0.655 (0.543, 0.762)	0.666 (0.540, 0.790)	0.662 (0.532, 0.780)	0.011 (−0.026, 0.060)	0.007 (−0.039, 0.048)