

Short Communication

## Improved prostate cancer detection with a human kallikrein 11 and percentage free PSA-based artificial neural network

Carsten Stephan<sup>1,\*</sup>, Hellmuth-Alexander Meyer<sup>1,a</sup>, Henning Cammann<sup>2</sup>, Terukazu Nakamura<sup>3</sup>, Eleftherios P. Diamandis<sup>4,5</sup> and Klaus Jung<sup>1</sup>

<sup>1</sup>Department of Urology, University Hospital Charité, D-10098 Berlin, Germany

<sup>2</sup>Institute of Medical Informatics, University Hospital Charité, D-10098 Berlin, Germany

<sup>3</sup>Department of Urology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan

<sup>4</sup>Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto M5G 1X5, ON, Canada

<sup>5</sup>Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto M5G 1L5, ON, Canada

\*Corresponding author

e-mail: carsten.stephan@charite.de

### Abstract

Human kallikrein 11 (hK11) was evaluated in a percentage free PSA-based artificial neural network (ANN) to reduce unnecessary prostate biopsies. Serum samples from 357 patients with (n=132) and without (n=225) prostate cancer (PCa) were analyzed and ANN models were constructed and compared to all parameters. The discriminatory power of hK11 was lower than that of PSA, but receiver operator characteristic (ROC) analyses demonstrated significantly larger areas under the curves for the ANN compared to all other parameters. ANNs with hK11 may lead to a further reduction in unnecessary prostate biopsies, especially when analyzing patients with less than 15% free PSA.

**Keywords:** artificial neural network (ANN); human kallikrein 11; prostate cancer; prostate-specific antigen.

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in the Western world (Jemal et al., 2005). Measurement of serum prostate-specific antigen (PSA or hK3) improves the early detection of PCa (Polascik et al., 1999). However, PSA measurement alone for detection of PCa lacks specificity, since non-malignant prostatic diseases such as benign prostatic hyperplasia (BPH) and chronic prostatitis also cause serum PSA elevation (Stephan et al., 2000). The detection of molecular forms of

PSA improves the specificity of PSA testing (Lilja et al., 1991; Stenman et al., 1991), in particular the use of percentage free PSA (%fPSA) (Catalona et al., 1998). To reduce the large number of unnecessary prostate biopsies (60–80%), the search for new markers and better methods of individual risk estimation are of utmost importance (Stephan et al., 2002c).

The human kallikrein 11 protein (hK11) is one of the possible new markers for better detection of PCa (Diamandis et al., 2002). It is encoded by the *KLK11* gene, which belongs to the human kallikrein family (Diamandis et al., 2000). hK11 is highly expressed in the prostate (Nakamura et al., 2001, 2003a). A hK11-specific immunoassay was developed and hK11 in seminal plasma and prostatic tissue extracts have been quantified (Diamandis et al., 2002). Elevated concentrations of hK11 were found in some PCa serum samples in preliminary investigations (Diamandis et al., 2002). These findings subsequently prompted another study on hK11 (Nakamura et al., 2003a). The authors found that serum hK11 concentrations were reduced in PCa patients. Furthermore, they showed that the hK11/tPSA ratio could contribute to better discrimination between BPH and PCa patients. The combination of %fPSA and hK11/tPSA could save ca. 50% of biopsies compared to %fPSA alone (Nakamura et al., 2003a).

Other approaches to improve the PCa detection rate are the use of different models of logistic regression (LR) (Carlson et al., 1998; Virtanen et al., 1999) and artificial neural networks (ANNs) using %fPSA together with PSA (Babaian et al., 2000; Finne et al., 2000; Djavan et al., 2002; Stephan et al., 2002b). In particular, ANNs have been increasingly used (Reckwitz et al., 1999; Veltri et al., 2002; Haese et al., 2003; Stephan et al., 2005a) because they have the ability to resolve complex non-linear relations among variables (Dreiseitl and Ohno-Machado, 2002).

The aim of this study was: (a) to evaluate hK11 as a single marker in comparison to PSA and %fPSA for the diagnosis of PCa; (b) to develop a %fPSA-based ANN using hK11, tPSA and age, and to evaluate it for diagnostic sensitivity and specificity; and (c) to study subgroups of patients for which the ANN may improve PCa detection or avoid unnecessary prostate biopsies. We wanted to exclude additional clinical information such as prostate volume or status of digital rectal examination to identify the diagnostic improvement of our ANN using only laboratory parameters and age.

The study included serum samples (drawn during 1998–2002) from 132 patients with PCa and 225 patients with histopathology-proven BPH from the Department of Urology, University Hospital Charité Berlin. The patients

<sup>a</sup>These authors contributed equally to this work.

Regarding a recommendation for future nomenclature of kallikrein gene-derived proteases, see the article 'A comprehensive nomenclature for serine proteases with homology to tissue kallikreins' by Lundwall et al., this issue pp. 637–641.

**Table 1** Descriptive statistics of various variables in serum from 225 BPH and 132 PCa patients (all patients, group I).

Variables	Median		Range		First quartile		Third quartile	
	BPH	PCa	BPH	PCa	BPH	PCa	BPH	PCa
hK11 ( $\mu\text{g/l}$ )	0.171 <sup>a</sup>	0.145	0.007–1.119	0.020–0.460	0.126	0.096	0.225	0.190
Age (years)	68 <sup>b</sup>	63	43–89	45–76	63	60	73	66.75
Total PSA ( $\mu\text{g/l}$ )	3.70 <sup>b</sup>	7.90	0.58–19.90	0.61–19.1	1.85	5.35	6.35	11.85
Free PSA (%)	17.10 <sup>b</sup>	8.40	2.10–80	1.90–56.6	11.65	5.63	22.25	12.63
hK11/total PSA	0.046 <sup>b</sup>	0.017	0.002–0.511	0.001–0.567	0.025	0.011	0.088	0.030
hK11/%free PSA	0.010 <sup>b</sup>	0.015	0.0003–0.091	0.0013–0.077	0.007	0.009	0.016	0.028
hK11/free PSA	0.290 <sup>a</sup>	0.203	0.017–4.762	0.008–5.788	0.147	0.120	0.593	0.367

Study groups and methods: the study included serum samples (drawn 1998–2002) from 132 patients with PCa and 225 patients with histopathology-proven BPH from the Department of Urology, University Hospital Charité Berlin, Germany. The patients have been described in detail previously (Stephan et al., 2002b, 2003). Total and free PSA were assayed using the IMMULITE PSA and Free PSA assays. The determination of hK11 concentrations was performed using an improved immunofluorometric serum assay (Diamandis et al., 2002) with a lower detection limit of 0.05  $\mu\text{g/l}$ . Of 357 samples, 14 (9 BPH and 5 PCa) had a hK11 value  $<0.05 \mu\text{g/l}$ . All calculations were performed with the original values. Recalculations with the values  $<0.05 \mu\text{g/l}$  set as 0.25  $\mu\text{g/l}$  did not change the results.

Statistical analyses: the statistical software package SPSS 12 for Windows (SPSS, Chicago, IL, USA) was applied using the Mann-Whitney U-test and the Spearman correlation coefficient. Significant differences are given as  $^a p < 0.05$  and  $^b p < 0.0001$  compared to PCa.

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Descriptive statistics for all parameters used are summarized in Table 1. Median values, ranges and lower and upper quartiles for hK11, age, tPSA, %fPSA, hK11/tPSA and hK11/%fPSA are given. Values for tPSA, %fPSA, age, hK11 and the ratios hK11/tPSA and hK11/%fPSA were all significantly different between PCa and BPH (Table 1). In contrast to PSA, hK11 was significantly lower in PCa patients compared to BPH patients. The hK11/fPSA ratio did not reach discriminatory power between PCa and BPH and is therefore only shown in Table 1.

hK11 showed a positive correlation with age ( $r_s = 0.25$ ;  $p < 0.01$ ), but with no other parameters. The %fPSA had a positive correlation with age ( $r_s = 0.265$ ;  $p < 0.01$ ) and showed a negative correlation with tPSA ( $r_s = -0.40$ ;  $p < 0.01$ ). All other parameters were not correlated with each other when analyzing all patients as one group.

Analyzing the PCa and BPH groups separately, hK11 did not correlate at all with tPSA or %fPSA. Again a positive correlation for hK11 with age was observed, but only in the BPH patients ( $r_s = 0.166$ ,  $p = 0.01$ ) and not in PCa patients. Lastly, the only negative correlation was observed between tPSA and %fPSA for all patients ( $r_s = -0.401$ ;  $p < 0.01$ ) as well as for the BPH ( $r_s = -0.227$ ;  $p < 0.01$ ) and PCa ( $r_s = -0.245$ ;  $p < 0.01$ ) subgroups.

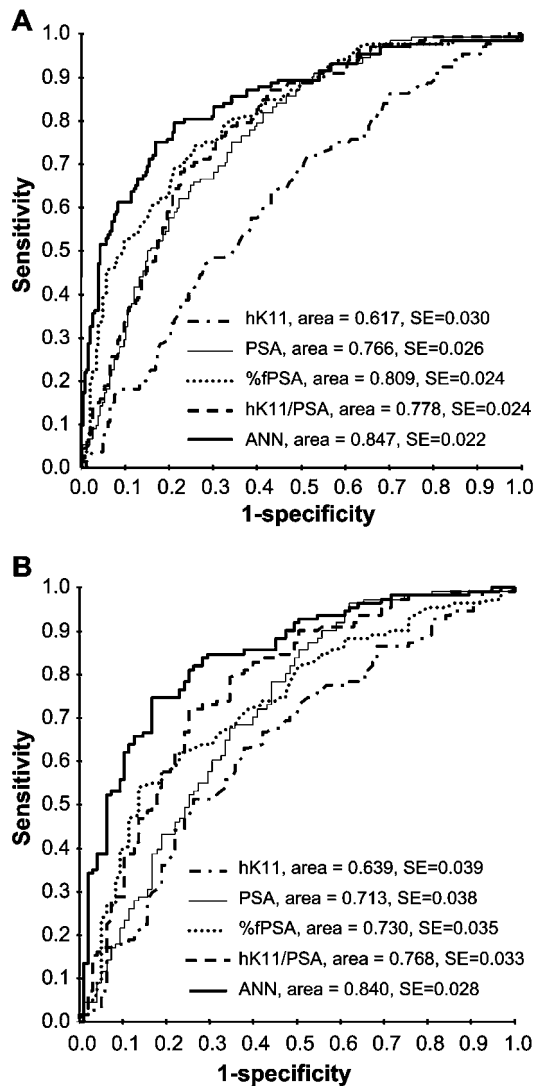
In a second step, data were analyzed for all patients ( $n = 357$ , group I) and for those patients with %fPSA values  $<15\%$  ( $n = 206$ , group II). For both groups, ANN models were constructed with the MATLAB Neural Network Toolbox (The Mathworks, Natick, MA, USA). The ANN outputs calculated with the four input variables tPSA, %fPSA, age, and hK11 were always significantly different

( $p < 0.0001$ ) in group I between all PCa patients ( $0.61 \pm 0.29$ ) and BPH patients ( $0.21 \pm 0.23$ ), as well as in group II between PCa ( $0.71 \pm 0.26$ ) and BPH ( $0.34 \pm 0.25$ ) patients. Receiver operating characteristic (ROC) comparisons were performed for all patients (group I, Figure 1A) and for group II (Figure 1B). The separate analysis of the subgroup was selected to show the potential to improve the diagnostic accuracy, since patients with %fPSA values  $<15\%$  usually undergo a prostate biopsy because of increased risk for PCa. We performed ROC analyses for all significantly different analytical variables and for ANN output values between PCa and BPH patients.

For the ANN calculation, different combinations of input factors were tested (e.g., exclusion of age, tPSA, %fPSA, or hK11). In group I, the ANN model with the input factors hK11, age, tPSA and %fPSA reached an area under the curve (AUC) of  $0.847 \pm 0.022$ . In group II the best ANN was achieved with an  $\text{AUC} = 0.840 \pm 0.028$  using all four input parameters. The exclusion of %fPSA, age or tPSA significantly reduced the AUC to 0.758, 0.779 and 0.792, respectively ( $p < 0.05$ ), while the exclusion of hK11 yielded an AUC value of 0.815 with a tendency ( $p = 0.08$ ) to a decreased diagnostic power.

To simplify evaluation of the diagnostic accuracy between the single parameters and the ANN results, only ANN models with the largest AUC including all four parameters were compared with the ROC data for the single parameters. Figure 1 shows that the ANN models significantly increased the diagnostic performance of the conventional parameter %fPSA in both groups (AUC for group I, 0.847 vs. 0.809,  $p = 0.022$ ; AUC for group II, 0.84 vs. 0.73,  $p < 0.0001$ ). Similarly, in group II, the ANN model clearly outperformed the best combination of hK11/tPSA (AUC for group I, 0.847 vs. 0.778,  $p = 0.001$ ; AUC for group II, 0.84 vs. 0.768,  $p = 0.013$ ).

For groups I and II, the diagnostic specificity and sensitivity obtained with tPSA, %fPSA, hK11, hK11/tPSA ratio (hK11/%fPSA ratio showed no additional clinical value, and was therefore omitted) and the ANN models at sensitivity and specificity of 90% are summarized in



**Figure 1** Receiver operating characteristic (ROC) curve analysis of hK11, tPSA, %fPSA, hK11/tPSA and ANN output for (A) all 357 patients (group I) and (B) for the 206 patients with an fPSA value of <15% (group II).

ROC curves were constructed and the area under the curve (AUC) was compared using GraphROC 2.1 for Windows (Kairisto and Poola, 1995) and MedCalc 8.1.1.0 (MedCalc Software). SE, standard error. For further details see the text.

Table 2. Data at other cutoffs such as 85% and 95% were also calculated (not shown). In all comparisons, the respective ANN models were always significantly better than hK11 alone ( $p < 0.01$ ). However, compared to %fPSA, the ANN only performed significantly better in group II ( $p = 0.012$ ) and not in group I ( $p = 0.76$ ) at 90% sensitivity. In both groups, the ANN performance was equal to hK11/tPSA at 90% sensitivity. However, in group II there was a slight increase in specificity at 90% sensitivity, from 47% for hK11/tPSA to 53% when using the ANN. The increased sensitivity at the 90% specificity level obtained with the ANN was obvious (Table 2, last two columns).

To summarize, the results of this study indicate that hK11 as an individual marker could not improve PCa detection compared with tPSA and %fPSA. However, the hK11-based ANN we developed using hK11, tPSA, age

and %fPSA performed significantly better compared with %fPSA and tPSA. Thus, approaches such as ANN models with the capability to significantly reduce prostate biopsies represent promising tools to overcome the dilemma of over-diagnosis and subsequent over-treatment of PCa in the PSA era (Stephan et al., 2005a).

Although hK11 levels were higher in prostate tissue compared with other tissues, and elevated concentrations were found in some advanced PCa serum samples in preliminary investigations (Diamandis et al., 2002), this study showed significantly ( $p < 0.05$ ) lower hK11 serum concentrations in PCa patients compared to BPH patients. Using double the number and other patients, these data confirm the findings of a previous study (Nakamura et al., 2003b). Until now, there has been a lack of comparative hK11 expression data between non-malignant and malignant prostatic tissue. The expression of hK11 in prostate cancer tissue was shown to be significantly reduced with increasing Gleason score and disease stage of the tumor (Stavropoulou et al., 2005), but that study did not include non-malignant tissue. On the other hand, our own studies on human kallikrein 2 and PSA proved that their tissue expression was down-regulated, whereas serum levels were increased (Magklara et al., 2000). Thus, a discordant relationship between tissue expression and levels in blood is possible, because the release of the components from tissue into the circulation is influenced by several factors (Stenman, 1997).

The ratio hK11/tPSA further enhances discrimination between PCa and BPH. However, in combination with markers that behave in a more linear unidirectional fashion, such as %fPSA, sensitivity and specificity may well be enhanced. For example, in BPH and PCa patients with a lower %fPSA value (<15%), consideration of the hK11 level might more accurately classify them into benign vs. malignant disease. Despite hK11 showing the weakest performance of all markers investigated, with a smaller AUC and lower specificity at the given cutoffs compared to tPSA and %fPSA, this parameter was of substantial value within an ANN. The ROC curve comparison revealed a significantly larger AUC for the hK11-based ANN than for %fPSA or hK11/tPSA. It is noteworthy that a parameter such as hK11 without correlation to tPSA or %fPSA might be more suitable for an ANN compared to another marker with a correlation to tPSA, such as hK2 (Stephan et al., 2005b).

It was previously demonstrated that the hK11/tPSA ratio can be used as a criterion to reduce the number of unnecessary biopsies in the subgroup of patients with %fPSA levels <20% (Nakamura et al., 2003a). We could confirm this by analyzing the hK11/tPSA ratio in group II. At 90% sensitivity, 47.4% of 95 BPH patients ( $n = 45$ ) could have avoided prostate biopsy. In addition, we were able to demonstrate that the ANN further enhances specificity in comparison to hK11/tPSA by 5.2% to 52.6%.

In conclusion, our results demonstrate that the combination of hK11, tPSA, age and %fPSA as parameters for an ANN could contribute to significantly better discrimination between BPH and PCa patients, especially for %fPSA <15%. The exclusion of prostate volume and status of digital rectal examination as clinical parameters within the ANN while retaining significantly improved

**Table 2** Specificity and sensitivity for tPSA, %fPSA, hK11, hK11/tPSA and the ANN at the 90% limits of sensitivity and specificity, respectively.

Parameter	Specificity at 90% sensitivity		Sensitivity at 90% specificity	
	Group I (n=357)	Group II (n=206)	Group I (n=357)	Group II (n=206)
Total PSA	48 (42–53)	44 (36–53) <sup>a</sup>	30 (22–38) <sup>c</sup>	22 (14–30) <sup>c</sup>
%fPSA	45 (40–51)	28 (21–37) <sup>a</sup>	52 (43–61)	40 (31–49)
hK11	19 (15–24) <sup>c</sup>	18 (13–27) <sup>b</sup>	18 (12–26) <sup>c</sup>	18 (11–26) <sup>c</sup>
hK11/tPSA	46 (40–52)	47 (38–56)	33 (25–42) <sup>b</sup>	29 (21–38) <sup>b</sup>
ANN	46 (40–52)	52 (44–61)	61 (53–70)	56 (46–65)

Sensitivity and specificity were calculated using the software MedCalc 8.1.1.0 (MedCalc Software, Mariakerke, Belgium). ANN models were constructed for both groups (group I, all patients; group II, patients with %fPSA values <15%) with the MATLAB Neural Network Toolbox. A feed-forward back-propagation network was applied, in which the input layer consisted of the variables tPSA, %fPSA, age and hK11. ANN models with a typical structure of three layers (input, hidden and output layers) were evaluated. Two neurons were used as hidden layers. Each ANN model was evaluated by the leave-one-out method (Stephan et al., 2005b). To obtain the best generalization of the ANN (i.e., to avoid overfitting) we used a routine that automatically sets the optimal performance function (Bayesian regularization) within the training run, as also used by Finne et al. (2000). The ANN output ranged from 0 (low PCa risk) to 1 (high PCa risk). The output values of the training run were then used to build the ROC curve. Values in parentheses are 95% confidence intervals.

Significant differences: <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$  and <sup>c</sup> $p < 0.0001$  compared with the ANN.

diagnostic accuracy is a notable finding when comparing these data to our previous studies (Stephan et al., 2002a). We used tPSA, %fPSA, age, prostate volume and status of digital rectal examination to generate the ANN named ProstateClass (Stephan et al., 2002a,b). This ANN model, which has been used in our clinic since 2002, is available free at: [www.charite.de/ch/uro](http://www.charite.de/ch/uro) (Stephan et al., 2002a). Although we have shown in this study that ANN models with hK11 as an additional input parameter reach a similar AUC compared to the ProstateClass ANN, further prospective settings are needed to evaluate the true power of hK11 as a new marker to avoid too optimistic a view for PCa diagnosis.

However, better biopsy pre-selection could separate those patients who truly need curative treatment instead of watchful waiting. On the other hand, biochemical failure of one-third further argues for the need of detection, especially of aggressive PCa. Patients with low %fPSA values, as in our group II with values <15%, tend to have more aggressive cancers (Stenman et al., 2005) and therefore these patients are of special interest for further cancer diagnostics.

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