

Review

New Markers and Multivariate Models for Prostate Cancer Detection

CARSTEN STEPHAN¹, HARRY RITTENHOUSE³, HENNING CAMMANN², MICHAEL LEIN^{1,4},
MARK SCHRADER¹, SERDAR DEGER¹, KURT MILLER¹ and KLAUS JUNG^{1,4}

¹Department of Urology and ²Institute of Medical Informatics, Charité - Universitätsmedizin Berlin, Germany;

³GenProbe Inc., San Diego, CA, U.S.A.;

⁴Berlin Institute for Urologic Research, Berlin, Germany

Abstract. *Specificity of PSA has been enhanced by using molecular forms of PSA and free PSA (fPSA) such as percent free PSA (%fPSA), proPSA, intact PSA or BPHA and/or new serum markers. Most of these promising new serum markers like EPCA2 or ANXA3 still lack confirmation of outstanding initial results or show only marginal enhanced specificity at high sensitivity levels. PCA3, TMPRSS2-ERG, and other analytes in urine collected after digital rectal examination with application of mild digital pressure have potential to preferentially detect aggressive PCa and to decrease the rate of unnecessary repeat biopsies. The combination of these new urinary markers with new and established serum markers seems to be most promising to further increase specificity of tPSA. Multivariate models e.g. artificial neural networks (ANN) or logistic regression (LR)-based nomograms have been recently developed by incorporating these new markers*

in several studies. There is generally an advantage to including new markers and clinical data as additional parameters to PSA and %fPSA within ANN and LR models. The results and unexpected pitfalls of these studies are shown.

With 186,320 new cases of prostate cancer (PCa) expected in the US in 2008, accounting for 25% of all cancers in men, this cancer has remained for more than 10 years as the malignancy with the highest incidence in the Western world (1). Without any doubt, prostate-specific antigen (PSA) plays the major role in the management of PCa. PSA values are directly related to a higher PCa probability (2-4) and no other marker currently replaces PSA as a first-line marker for PCa diagnosis. However, other non-malignant conditions of the prostate such as benign prostate hyperplasia (BPH) can also increase serum PSA and are often responsible for elevated PSA values in the absence of biopsy-detected PCa (5). On the other hand, several studies have shown that a single elevated PSA value (6, 7) or the PSA velocity as PSA increase over time (8, 9) can detect PCa with clinical sensitivities in the presence of other benign diseases of the prostate. The specificity of PSA remains problematic. With a positive PCa detection rate of 20-40% in initial biopsies, the dilemma of low specificity and a low positive predictive value of only 25% in a pooled meta-analysis at the cutoff >4.0 µg/L was clearly shown (10). This results in many unnecessary biopsies. Although there is a need to improve PCa detection it is even more important to detect aggressive and life threatening cancers and to stratify less-aggressive PCa which may best managed with less-aggressive therapy or active surveillance. The wide-spread use of PSA to detect early PCa has led to a serious over-treatment problem (11). On the other hand, the continuous mortality from PCa demonstrates delayed detection in many patients. Results of two large randomized trials in Europe and the USA are awaited in 2009/10 to show if PSA screening can reduce PCa-related mortality. The additional

Abbreviations: ANN, Artificial neural network; ANXA3, annexin A3; AUC, area under receiver operating characteristic curve; BPH, benign prostatic hyperplasia; BPHA, BPH associated (fPSA); cav-1, caveolin-1; cPSA, complexed PSA; DRE, digital rectal examination; EPCA, early PCa antigen; fPSA, free PSA; inPSA, intact PSA; IGF-1, insulin-like growth factor 1; KLK, human kallikrein; LOO, leave-one-out; LR, logistic regression; MIC-1, macrophage inhibitory cytokine 1; MIF, macrophage migration inhibitory factor; NEM, no evidence of malignancy; PCa, prostate cancer; PCA3, prostate cancer antigen 3; PSA, prostate-specific antigen; PSP94, prostatic secretory protein 94; %fPSA, percentage free PSA; tPSA, total PSA; proPSA, precursor form of PSA; p2PSA, (-2)proPSA; ROC, receiver operating characteristic.

Correspondence to: PD Dr. Carsten Stephan, Department of Urology, Charité - Universitätsmedizin Berlin, CCM, Charitéplatz 1, D 10117 Berlin, Germany. Tel: +49 30450615159, Fax: +49 30450515904, e-mail: carsten.stephan@charite.de

Key Words: PSA, PCA3, prostate cancer, ANN, review.

performance of a digital rectal examination (DRE) is routine in urological practice because some PCa can be detected only with a suspicious DRE but comparing DRE and PSA shows a clear advantage for PSA (12).

PSA remains the gold standard for staging or for detection of biochemical recurrence in PCa patients. To date, no other test shows the power to predict advanced or metastatic PCa. An excellent review summarizing PSA screening studies and the role of PSA for recurrence was published recently by Lilja *et al.* (13).

PSA-based analysis such as PSA velocity, PSA density or PSA doubling time have been extensively summarized (14, 15). However, a multivariate model incorporating PSA velocity and % fPSA was not successful at improving PCa detection (16).

Data from valid tissue markers such as hepsin, prostate stem cell antigen, prostate-specific membrane antigen, AMACR and others such as proteomic pattern for PCa have been recently summarized elsewhere (17). AMACR is currently used in biopsy tissue as a molecular pathology parameter with p63 and cytokeratin as part of the triple-antibody cocktail for PCa detection (18).

Silencing of the glutathione-*S*-transferase pi (GSTP1) gene by methylation is a frequent somatic genome alteration reported in PCa and high grade prostate intraepithelial neoplasia tissue (19). Data on GSTP1 hypermethylation in urine sediment and serum have been inconsistent and has been reviewed by others (20, 21). The use of PCa tissue markers in multiplex urine analysis have been introduced recently (22, 23) and the most interesting parameters will be discussed later. Possible mechanisms of molecular alterations in PCa such as upstream epigenetic changes and genetic polymorphisms to downstream modulations through alternative splicing and other post-translational processes have been recently reviewed (24).

One of the most transforming discoveries in many years has been the identification of gene fusion (translocation) of the nuclear transcription factor genes *ERG* and *ETV1* with the 5'DHT regulated promoter *TMPRSS2* (25). The frequency of this PCa specific gene fusion represents approximately 50% of PSA-screened PCa (26). *TMPRSS2* is an androgen-regulated gene and is more commonly associated with Gleason scores >7 (41% *versus* 12%) and more PCa deaths and/or metastatic disease development (53% *versus* 23%) (27). A urinary test for the detection of *TMPRSS2:ERG* fusion products has been developed using RNA amplification and quantitative PCR (28). Apart from being promising biomarkers, these fusion genes may be important for prognosis and treatment selection. The gene-fusion products are now candidates as targets for therapy. Important new data on this topic have also been reviewed (29).

New serum and urine markers and their application in multivariate models will be described below.

Serum Markers

Free PSA and PSA complexes. In the early 1990s, two independent groups discovered that PSA exists in different molecular forms, such as free (unbound) PSA and PSA bound to alpha1-antichymotrypsin (PSA-ACT) (30, 31). In general, 65-95% of the PSA is PSA-ACT, whereas free PSA represents, on average, only 5-35% of the total PSA (tPSA). The relative amount of fPSA tends to be increased in benign prostate hyperplasia (BPH) compared with PCa. Possible explanations for these differences, including the clinical impact on the ratio of free PSA (fPSA) to PSA (% fPSA), have been reviewed (13, 32-34). The % fPSA can avoid approximately 20-25% of unnecessary biopsies in the PSA range of 4-10 µg/L (35, 36). To date, % fPSA is the only accepted routine reflex assay with PSA.

At least two other PSA complexes are also known to exist, PSA-alpha2-macroglobulin (PSA-A2M) and PSA-alpha1-protease inhibitor (PSA-API) (37, 38). Their clinical relevance is limited due to the complicated technology required to measure the PSA-A2M complex or due to the relatively small amount of PSA-API related to total PSA (tPSA). PSA assays do not detect the complex with A2M. The sum of PSA-ACT and PSA-API is termed as complex PSA (cPSA) and can be assayed by using a blocking antibody against fPSA (39) but this method does not detect the PSA-A2M complex. It had been initially proposed that cPSA can be used as a single marker instead of % fPSA (40) but only the ratio cPSA/tPSA can reach equivalent results compared with % fPSA (41, 42). Possibly cPSA can be used as the initial marker with a small advantage compared with tPSA.

ProPSA. PSA is initially synthesized as a pre-proprotein with 261 amino acids. The removal of an amino-terminal leader generates a 244 amino acid non-catalytic proform of PSA named proPSA. The removal of the 7-residue propeptide generates the catalytically active fPSA form, a 237-residue single-chain enzyme. ProPSA is a distinct molecular form of fPSA. fPSA exists in blood as a mixture of enzymatically inactive forms including several truncated proPSA forms with the propeptide *N*-terminally truncated at various positions (43, 44). These different proPSA forms contain 1-5 amino acids in the proleader peptide instead of the native 7 amino acids and were described as (-2)proPSA and (-4)proPSA. In marked contrast to the rapid activation of the native (-7)proPSA, the truncated (-2)proPSA is not activated and accumulates in prostate tissue.

The (-7) and (-5)proPSA forms are detected by a research assay (Roche Diagnostics, Penzberg, Germany) with limited success to distinguish PCa from BPH and to separate aggressive and non-aggressive PCa (45, 46). However, in a study of 376 men with PCa undergoing prostatectomy, the ratio (-5, -7)proPSA/% PSA was associated with higher

Gleason grade ($p=0.001$) and non-organ-confined PCa ($p<0.0001$) (47).

Based on early promising results with a combination of (-2) proPSA and (-4)proPSA in an assay (Beckman Coulter, San Diego, CA, USA) regarding the discrimination of PCa and patients with no evidence of malignancy (NEM) (48) and detection of aggressive PCa (49), there is now an automated research assay available measuring only the (-2)proPSA (p2PSA). New data show that p2PSA/fPSA (named % p2PSA) had the greatest area under the ROC curve (AUC) with 0.69 followed by % fPSA (AUC: 0.61) (50). In addition, in the 2-10 µg/L tPSA range the % p2PSA had the largest AUC of 0.73 (50). Both proPSA assays, the (-5, -7)proPSA and (-2) proPSA have been included in multivariate models and data will be shown below. Helpful reviews on proPSA have been published (51, 52).

BPSA (BPHA). BPSA (named BPH associated: BPHA) is another form of fPSA that is identical to native PSA containing 237 amino acids like PSA, but with 2 internal peptide bond cleavages at Lys182 and Lys145, which inactivates PSA and prevents it from forming complexes with protease inhibitors (53, 54). BPSA is correlated with benign disease and specifically with clinically benign prostatic hyperplasia (55, 56).

A review focused on BPSA has been published (57). In line with the recently available automated p2PSA assay, BPSA can also be measured on the same analyzer (Access analyzer, Beckman Coulter). Recent data in 541 histologically proven PCa and NEM patients showed an improved discrimination between BPH and PCa for BPHA/tPSA (AUC 0.69) when compared with tPSA alone (AUC 0.59) but % fPSA had the largest AUC at 0.775 (58).

A combination of both assays, the p2PSA and BPHA on the same analyzer, may be more advantageous than a combination of (-2)proPSA, (-4)proPSA and BPSA (59).

Intact fPSA. An additional form of fPSA is intact, non-native PSA (inPSA), which has been identified in tissue and serum (60). inPSA is not internally clipped but is inactive PSA that does not form complexes with serum proteins. In a simplified fPSA model, inPSA can be calculated by subtracting BPSA and the sum of all proPSA forms from fPSA (51). The fPSA in serum contains, on average, roughly equal proportions of the 3 molecular forms. However, the relative amounts of proPSA and BPSA vary significantly in individual patients. The proportion of inPSA in serum is elevated in patients with NEM and tends to be the inverse of the proPSA concentration. A possible explanation for the correlation of free PSA with benign tissue is the combined amounts of BPSA and inPSA (32).

Another specific monoclonal antibody to a free PSA form, fPSA-I, detects only the non-clipped forms of free PSA (61).

Thus, the fPSA-I assay would be expected to detect both proPSA and inPSA but not BPSA. Studies with fPSA-I have yielded encouraging results for PCa detection (62, 63). However, as yet no commercial assay for fPSA-I is available and it is questionable if the sum of proPSA and inPSA, which theoretically equals the difference of fPSA and BPSA, can be a better tumor marker than the more PCa-specific proPSA alone.

It should be noted that all of these PSA forms are worthy of further research to determine whether they can improve the accuracy of PCa detection. Initial results are mostly promising and a combination into a panel of markers or multivariate models with some of these markers may further improve PCa detection. Since the mechanism of release into blood is the same for all PSA isoforms *i.e.* disruption of the prostate architectural integrity, molecular markers with a distinctly different mechanism, and the direct detection of PCa cells in urine are predicted to be more synergistic. Nomograms with combinations of PSA and PCA3 have already demonstrated synergy.

With the exception of proPSA, all other molecular forms of fPSA failed to demonstrate consistently improved specificity over PSA and % fPSA for PCa detection. A better detection of BPH may not be a goal since patients often have both PCa and BPH concurrently.

The kallikrein family. Within the last decade, the human kallikrein family has been identified, which contains at least 15 genes (64). From the initially known 3 kallikreins KLK1 to KLK3, the human glandular kallikrein 2 (KLK2) and especially KLK3 (PSA), have been used as PCa serum markers. A new nomenclature for all kallikrein genes simplified their classification as *KLK1* to *KLK15* encoding for the proteins KLK1 to KLK15 (65). Beside PSA, KLK2 has been shown to add information for detecting PCa (66, 67) but this marker is not used routinely for PCa detection nor exists as a commercially available assay. Research assays of KLK4 or KLK15 show only marginal success in PCa diagnosis. Only KLK11 improves specificity of tPSA or % fPSA in combination with both parameters (68). Due to close relationships and similarities of all kallikreins and differential expression of several kallikreins in PCa tissue, applications for prognosis may be possible (17).

MIC-1. An immunoassay in 1,000 men for macrophage inhibitory cytokine 1 (MIC-1) showed it to be an independent predictor of the presence of PCa and tumors with a Gleason score >6 (69). A diagnostic algorithm (MIC-PSA score) based on serum levels of MIC-1, tPSA and % fPSA potentially reduced the number of unnecessary prostate biopsies by 27% (69). Data from another study showed also significant lower MIC-1 serum concentrations for PCa compared with BPH but the AUC for MIC-1 reached only 0.6 and was lower than the AUC for PSA (0.64) and % fPSA (0.69), respectively (70).

MIF. Another marker, the cytokine macrophage migration inhibitory factor (MIF), was elevated in serum of PCa patients compared with BPH patients (71). MIF strongly correlated to tPSA ($r_s=0.61$) in 509 analyzed patients, but unfortunately a histological diagnosis of PCa was only available in 152 patients, making interpretation of these data difficult (71). Another group could not confirm these data in 213 PCa and BPH patients, showing no correlation of MIF to tPSA ($r_s=-0.05$). In addition, MIF values were in fact decreased and not significantly higher in PCa (72). A third study using the MIF assay also found decreased MIF values in PCa compared with BPH and a low AUC of 0.54 for MIF (70).

Caveolin. An immunoassay with specificity for caveolin-1 (cav-1), the major protein component of caveolae, which appear to mediate molecular transport, cell adhesion and signal transduction activities in a cell, showed highest concentrations in 102 PCa patients (median: 0.463 $\mu\text{g/L}$) compared with 81 healthy men (0.324 $\mu\text{g/L}$) and 107 BPH patients (0.172 $\mu\text{g/L}$) (73). Unfortunately, PSA was not available for all patients (73). The same group measured cav-1 in serum of 419 PCa patients with a median serum level of 1.01 $\mu\text{g/L}$ being 2-fold higher than in the previous study (74). However, data are yet to be confirmed.

EPCA. An assay for a nuclear matrix protein in serum, the so-called early PCa antigen (EPCA) showed highly promising values with 92% diagnostic sensitivity and 94% specificity in a small cohort of 46 patients including 12 PCa (75). A study performed with a larger cohort of 385 men resulted in a 92% diagnostic specificity and a 94% sensitivity with an AUC of 0.89 (76). However, methodological deficiencies have been identified and the validation of this marker by other researchers is needed (77). Analysis of a second EPCA epitope, the EPCA-2.19 revealed in 328 patients a 100% specificity at 91% sensitivity with an AUC of 0.98 (78). Independent studies have not yet been completed.

IGF. High serum levels of insulin-like growth factor 1 (IGF-1) have been retrospectively shown to be associated with the future development of PCa (79). However, this could not be confirmed in screening studies (80, 81). In plasma, most IGF-1 is bound to several binding proteins, of which IGFBP-3 is the major one. However, adjustment for IGFBP-3 has not been found to modulate the impact of IGF-1 on the PCa risk (79). A recent meta-analysis of 12 prospective studies in ~3700 men indicated that high circulating IGF-I concentrations are associated with a moderately increased risk for prostate cancer (82).

PSP94. While an investigation in 1993 found no differences between PCa, BPH and controls in serum concentrations of prostatic secretory protein of 94 amino acid residues (PSP94) (83), a recent study in 1,212 men (49.2% with

PCa) using a sandwich enzyme-linked immunosorbent assay showed significantly lower values in PCa (84). Among a subgroup of 649 men with lower PCa risk, PSP94 could discriminate aggressive PCa from moderate grade disease (Gleason score 7) and low grade disease ($p=0.007$) (84). PSA and %fPSA were not able to distinguish between patients with different grades in this group.

Multivariate analysis showed that PSP94-binding protein (PSPBP) and the bound/free PSP94 ratio were independent predictors of biochemical relapse following radical prostatectomy (85).

Urine Markers

Annexin A3. Annexin A3 (ANXA3) is a calcium-binding protein with lower production in PCa than in BPH, prostate intraepithelial neoplasia, and healthy tissues (86). ANXA3 in tissue was also able to stratify 969 PCa intermediate-risk patients into high- and low-risk subgroups (87). Quantification by ANXA3 using Western blots of 591 urine samples showed significantly lower values in PCa compared with BPH patients, with low specificities at high sensitivities but an improved sensitivity at high specificities compared with tPSA (88). However, the combination of ANXA3 and PSA (AUC 0.81) had higher AUC compared with %fPSA (0.73) (88).

PCA3. Prostate cancer antigen 3 (PCA3), also known as DD3, is a non-coding RNA produced almost exclusively in the prostate and has been shown to be 66-fold overexpressed in PCa tissue compared with normal prostate tissue (89). The commercially available test APTIMA[®] (Gen-Probe) uses transcription mediated amplification (90) and in a large European prospective multicenter study clearly demonstrated a greater probability of a positive repeat biopsy with increasing PCA3 scores (91). In those 463 men, the PCA3 score was significantly higher in men with Gleason score ≥ 7 versus < 7 , and “significant” versus “indolent” PCa indicating a possible role to predict aggressiveness and stage (91). Furthermore, PCA3 has the advantage compared with PSA that it is independent of prostate volume (91), correlates to tumor volume (92, 93) and may even predict extracapsular tumor extension (93). In a recent study of a small population, data from 62 prostatectomy specimens showed a trend of PCA3 to correlate with expression of the progression marker EZH2 (94).

Methylation markers. A multicenter study of DNA methylation markers in urine-based assays with a clinical prototype has recently been published (23). This multiplexed, quantitative methylation-specific PCR assay consisting of the 3 methylation markers, GSTP1, RARB and APC, and an endogenous control, was tested in 234 patients from 9 clinical sites (23). In two independent cohorts, where the multiplexed assay was combined with serum tPSA, DRE

status and age, the AUCs of 0.69 and 0.65 showed only marginal overall success (23). However, a good correlation of GSTP1 with the number of tumor-positive or suspicious cores on biopsy or higher tumor volumes in samples that exhibited methylation for either *GSTP1* or *RARB* indicated some potential use as an aid for prostate biopsy (23).

GOLM 1. *GOLM1* is consistently up-regulated in PCa and has been demonstrated at the protein level. Prostate epithelial cells were identified as the cellular source of *GOLM1* expression using laser capture microdissection technique (95). *GOLM1* transcript levels in urine sediments (AUC 0.62) were a significant predictor of PCa outperforming PSA (AUC 0.495) (95). This indicates that up-regulation of *GOLM1* expression and its appearance in patients' urine has potential as a novel biomarker for clinically localized PCa.

Simultaneous measurement of potential discriminative urine metabolites including sarcosine and combined analysis with other markers should be investigated (96, 97). The recent upsurge in studies of molecular assays for PCa cells in urine and metabolites associated with PCa in urine highlight the value of this specimen to provide increased specificity and prognostic value for PCa compared with current serum immunoassays.

Multivariate Models with Available New Markers

When estimating the diagnostic validity of the single tumor marker PSA it is obvious that lower thresholds increase sensitivity with the caveat of lower specificity. Consequently, this results in more unnecessary biopsies. Higher cutoff levels increase the specificity but many PCa are missed. One approach to predict the likelihood of a positive prostate biopsy is to combine PSA and other markers with classification models like logistic regression (LR)-based nomograms, artificial neural networks (ANN), classification and regression tree analyses and risk-group systems. Nomograms have been widely utilized for different indications to detect PCa, predict recurrence, extracapsular extension or lymph node invasion compared with other classification models (98). Nomograms show advantages compared with the PCa risk calculator, and PSA alone (99). Some author groups concluded in summaries (98, 100) and others in comparison studies (101-103) that nomograms are more accurate and have better performance characteristics than other alternatives. In contrast, several other studies (104-106) and reviews (107, 108) showed advantages for ANN compared with LR-based nomograms. Disparate results from the same group favoring LR (102) or ANN (105), or lack of advantage for LR when considering different PSA assays (101, 109) show that ambivalent results may be due to population or input parameter differences rather than true methodological differences. When comparing both LR and ANN in large populations (>5,000), they tie in 7 out of 8

studies (108). This strongly supports the notion that there is no mathematical advantage for either method. A summary on the differences and similarities of these models from a technical point of view of both LR and ANN and further classification models such as linear discriminant analysis, support vector machines or nearest neighbour classifier can be recommended for further details (107).

In the following pages most of the already mentioned new markers were analyzed regarding their performance in LR and/or ANN models.

PSA, %fPSA and cPSA based multivariate models. After initiation of ANN models into PCa diagnostics in 1994 by Snow *et al.* (110) one important further improvement was the introduction of %fPSA into clinical routine use (36). Soon after, several LR and ANN studies incorporating %fPSA showed a further improvement of up to 50% specificity compared with %fPSA alone (104, 105, 111, 112). A recent review summarizes all these studies with additional aspects of commercial PSA assays (113). A valuable comparison of all available ANN and LR studies to detect PCa has been recently published by Schröder and Kattan (114).

An ANN was published in 2003 from a model based on 7 clinical features including age, race, family history, International Prostate Symptom Score, DRE, tPSA and cPSA (106). An evaluation of 5 different tPSA assays including fPSA or cPSA in 780 patients found similar high AUC for the ANN model with cPSA/tPSA (0.89) compared with those models including %fPSA (AUC 0.88-0.89) (115). The ANN for those 5 different tPSA assays is freely available online (<http://www.charite.de/pcaberlin/ann5/ann5.html>) and differences in this study are mostly based on assay variations and not on population variances, which limited other earlier ANN and LR comparisons (116, 117). Especially for the use of cPSA in a limited tPSA range, a new method called discordance analysis characteristics (DAC) promises to be useful in improving PCa detection (118). A view of this program is given in Figure 1.

Free PSA subforms in multivariate models. A study on a relatively small population using the (-5, -7)proPSA and KLK2 showed only marginal success when using LR to distinguish between BPH and PCa (45). Another investigation in 898 patients from two centers showed only in the referred group in the 4-10 µg/L tPSA range that a leave-one-out (LOO) ANN model including the two variables %fPSA and (-5, -7) proPSA could reach the same performance as a routinely used ANN with PSA, %fPSA, age, prostate volume and DRE (119). However, at 95% sensitivity the ANN could not improve specificity compared with %fPSA (119).

The (-2)proPSA assay measured with an automated research assay (named as p2PSA) showed in an initial study

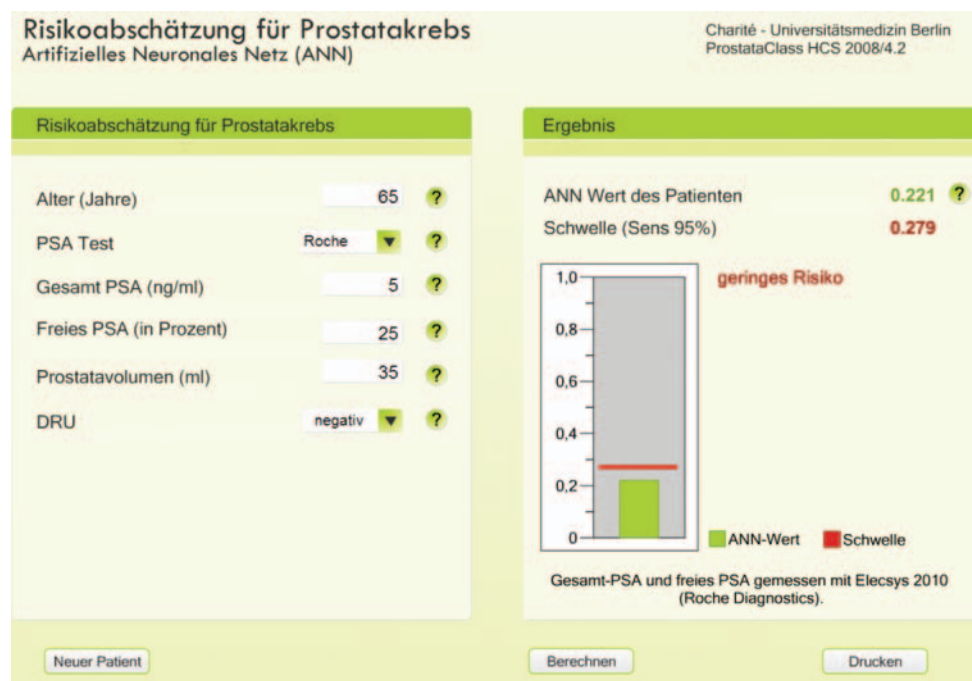


Figure 1. "ProstataClass" program (German version) with age, PSA assay (5 possibilities), total PSA, percent free PSA, prostate volume and status of digital rectal exam as input factors. Example of the ANN output indicating lower risk at the 95% sensitivity level.

in 123 men that the LR model with %p2PSA had the greatest AUC compared with %fPSA and %p2PSA (50). However, this advantage diminished in the 2-10 µg/L tPSA range, with equal AUC (0.73) for %p2PSA and the LR model (50).

The first study with the new automated p2PSA assay in combination with LR and ANN models in 586 patients by using tPSA, %fPSA, %p2PSA and age but not prostate volume showed the highest AUCs for the LOO ANN and LR model (0.85 and 0.84) and best specificities (ANN: 62.1% and 45.5%; LR: 53.1% and 41.2%) compared with tPSA (22.7% and 11.4%) and %fPSA (45.5% and 26.1%) at 90% and 95% sensitivity (120). These ANN models also did not require prostate volume like the (-5, -7)proPSA-based ANN (119) but the advantage over %fPSA was consistently significant at both 90% and 95% sensitivity. Furthermore, %p2PSA was better than tPSA and %fPSA at distinguishing between pT2 and pT3, and Gleason score <7 and ≥7 PCa, indicating a possible role in the preferential detection of aggressive and clinically significant tumors (120).

Data from the first study on the automated BPHA assay and an ANN application showed a further improved discrimination between BPH and PCa when comparing %fPSA (AUC: 0.775) or BPHA/tPSA (AUC 0.69) with a LOO ANN (AUC: 0.81) with Bayesian regularization utilizing the input factors age, prostate volume, tPSA, %fPSA and BPHA/tPSA (58). Within the 0-10 µg/L tPSA

range the ANN model with BPHA/tPSA also reached the best specificities (53.9% and 44.5%) compared with the ANN without BPHA/tPSA (50% and 40.6%) and %fPSA (40.9% and 27.2%) at 90% and 95% sensitivity. Incorporation of BPHA/tPSA into an ANN model increased the specificity compared with %fPSA by 13% and 17%, at 90% and 95% sensitivity (58). Possibly, the automated BPHA assay can be used as an additional tool to improve PCa detection for instance in combination with p2PSA by incorporating those new markers into an ANN.

One survey with fPSA-I using a logarithmic combination of fPSA-I, %fPSA and tPSA showed only a slightly higher, but non-significant AUC (0.773) compared with fPSA-I/tPSA (0.755) (63). In the 2-10 µg/L tPSA subgroup only the AUC of logarithmic fPSA-I, %fPSA and tPSA (0.706) and fPSA-I/tPSA (0.704) were significantly larger compared to the AUC of tPSA (0.602) (63).

A panel of the kallikrein markers with %fPSA, intact PSA and KLK2 in a screening cohort of 740 men improved the AUC from 0.68 to 0.83 compared with a model including only age and PSA (121). Multiple kallikrein forms measured in blood may predict the result of the biopsy.

Kallikreins and other serum markers in multivariate models. The incorporation of other kallikreins into PSA and %fPSA based ANN was first performed with KLK2, which only slightly increased specificity over %fPSA (45, 122). At the

low tPSA range 1-4 µg/L, the KLK2 and %fPSA ANN model was also able to significantly improve specificity at high sensitivity cutoffs compared with %fPSA, but this was not possible within the tPSA range 4-10 µg/L (122).

After initial promising results on KLK11, which showed improved specificity in combination with %fPSA (68), another subsequent study again showed marginal improvement of PCa differentiation from BPH when using a %fPSA and KLK11 based ANN (123). While the AUC with 0.84 for the ANN was significantly larger than for %fPSA, significantly better specificities at 90% sensitivity for the ANN compared with tPSA and %fPSA but not KLK11/tPSA could only be reached in a subpopulation with a higher PCa risk and %fPSA values <15% (123).

Incorporation of the other already mentioned new serum markers MIC-1 and MIF into the %fPSA and KLK11 based ANN showed an improved AUC (0.86) compared with %fPSA (0.81) but no difference at 90 or 95% sensitivity (70). However, when also including prostate volume, the advantage for the ANN with KLK11, MIC-1 and MIF compared with %fPSA was always visible (70). Application of kallikreins and their role in multivariate models and other biomarkers have been reviewed extensively (17, 113, 124, 125).

Two studies have been published on IGF-1 as input factor in a multivariate model (126, 127). Whereas the discriminatory value of KLK2 and IGF-1 alone was absent between PCa and NEM patients within the diagnostic “gray zone”, the KLK2/fPSA ratio at tPSA 4-10 µg/L and the IGF-1/fPSA ratio at tPSA >10 µg/L showed the largest odds ratio in multivariate analysis. However, for all 345 patients, %fPSA had the best odds ratio (126). The AUC comparison showed a slight advantage for KLK2/fPSA (0.74) and IGF-1/fPSA (0.75) compared with %fPSA (0.71) at tPSA 4-10 µg/L (126). A similar improvement in AUC for IGF-1/fPSA (0.75) compared with %fPSA (0.69) was observed in another study of 586 patients at tPSA 4-10 µg/L (127). An LR model confirmed the higher potential of the IGF-1/fPSA ratio to discriminate benign prostate disease from PCa (crude odds ratio for IGF-1/fPSA: 4.74) and at 90% sensitivity IGF-1/fPSA had a 10% higher specificity compared with %fPSA (127).

Multivariate analysis of PSP94 in a study with 1,212 men showed an improved AUC (0.74) for a model with PSP94 and %fPSA compared with the baseline model with age, DRE, ethnic background and tPSA (AUC: 0.66) and those models with PSP94 or %fPSA alone (each 0.72) (84). Further, among a subgroup of 649 men with tPSA <20 µg/L, the model with both PSP94 and %fPSA (AUC: 0.75) clearly improved performance of the baseline models with tPSA (0.56), %fPSA (0.64) or PSP94 alone (0.67) (84).

Use of urine marker PCA3 in novel multivariate models. Recently, first results on the combination of PCA3 and the *TMPRSS2-ERG* gene fusion product have been presented

(128, 129). The first study combined both urinary analytes in 108 patients (129). While in the 78 PCa patients the semi-quantitative RT-PCR analysis followed by hybridization for the *TMPRSS2-ERG* detection had a sensitivity of 37%, the PCA3 assay had a sensitivity of 62% and the combination of both parameters increased sensitivity to 73% (129). More importantly, in those men with persistently elevated tPSA levels and a history of negative biopsies the positive predictive value of *TMPRSS2-ERG* fusion transcripts was 94% (129).

The second study had data available from serum PSA in addition to the urinary measured PCA3 and the *TMPRSS2-ERG* in 105 patients (128). PCA3 alone had an AUC of 0.65 when comparing the 32 PCa and 73 NEM patients, while the combination of PCA3 and *TMPRSS2-ERG* increased the AUC to 0.77. Further addition of serum PSA to PCA3 and *TMPRSS2-ERG* resulted in an AUC of 0.80 (128). At 67% sensitivity, 77% specificity was achieved when combining all three input parameters. Both the LR and individual cut-off approaches produced the same sensitivity and specificity results.

Data from both studies showed synergy between PCA3 and *TMPRSS2-ERG* assays for predicting biopsy outcome and suggest that further improvement in diagnostic accuracy could be achieved using LR-based nomograms that also incorporate serum PSA.

There might be more promising results when incorporating also other serum parameters such as %fPSA or p2PSA into urinary-based measurements of PCA3 and *TMPRSS2-ERG* to detect PCa. Prospective data of 252 PCa patients with 9 years of follow-up indicated that the *TMPRSS2:ERG* fusion was highly associated with aggressive PCa (Gleason score >7) and PCa-related death (27). Thus, PCA3 and *TMPRSS2-ERG* do have a potential to be used, especially to detect aggressive and life-threatening PCa.

Multivariate regression analysis in a multiplexed model, including SPINK1, PCA3, GOLPH2 and *TMPRSS2:ERG* showed significantly greater AUC (0.758) than PCA3 (0.662) or serum PSA (0.51) alone (22).

The other recent multiplexed survey with the 3 methylation markers, GSTP1, RARB and APC in urine showed an improved AUC of 0.65 for the multivariate combination of all 3 markers, tPSA, DRE status and age compared with tPSA (0.54), the 3 markers together (0.6), and the clinical data tPSA, DRE and age together (0.6) (23). However, the AUC increase was not statistically significant (23).

Conclusion

The use of %fPSA and especially the incorporation of this ratio into multivariate models increased the specificity of PSA in the last decade. Of all molecular forms of fPSA, (-2)proPSA measured with an automated p2PSA assay most likely further improves the specificity of %fPSA but only within LR and ANN models. After initial promising results

especially for the kallikrein KLK2, no marker of the kallikrein family besides PSA (KLK3) has been used so far in the clinical routine diagnosis of PCa. Of all new serum markers, EPCA2 had the most promising initial results, with extremely high sensitivities and specificities, but these findings are yet to be confirmed. PCA3 as a urine marker has the potential to detect PCa in previous biopsy-negative patients and especially to detect aggressive PCa. Here, most studies from different working groups are in line with the first positive data. The combination of *TMPRSS2-ERG* gene fusion data, PCA3 or other new urine markers and serum markers should be tested in the future as one of the most promising models to further increase the specificity of tPSA. A combination of the results from individual assays for PCA3, gene fusion and PSA forms may enable significantly improved management of PCa including early detection, prognosis and treatment selection. This review also showed that critical examination of LR-based nomograms and ANNs designed to predict the risk of a positive prostate biopsy for PCa is necessary and that, to date, both methods should be used in preference to single markers or other classification models.

Acknowledgements

This work was partially supported by the DFG (STE 996/2-1) and the SONNENFELD-Stiftung.

References

- Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics, 2008. *CA Cancer J Clin* 58: 71-96, 2008.
- Aus G, Damber JE, Khatami A, Lilja H, Stranne J and Hugosson J: Individualized screening interval for prostate cancer based on prostate-specific antigen level: results of a prospective, randomized, population-based study. *Arch Intern Med* 165: 1857-1861, 2005.
- Postma R, Schroder FH, van Leenders GJ, Hoedemaeker RF, Vis AN, Roobol MJ and van der Kwast TH: Cancer detection and cancer characteristics in the European Randomized Study of Screening for Prostate Cancer (ERSPC) – Section Rotterdam. A comparison of two rounds of screening. *Eur Urol* 52: 89-97, 2007.
- Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, Minasian LM, Ford LG, Lippman SM, Crawford ED, Crowley JJ and Coltman CA Jr: Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. *N Engl J Med* 350: 2239-2246, 2004.
- Stamey TA, Caldwell M, McNeal JE, Nolley R, Hemenez M and Downs J: The prostate-specific antigen era in the United States is over for prostate cancer: What happened in the last 20 years? *J Urol* 172: 1297-1301, 2004.
- Schroder FH, Roobol MJ, van der Kwast TH, Kranse R and Bangma CH: Does PSA velocity predict prostate cancer in pre-screened populations? *Eur Urol* 49: 460-465, 2006.
- Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, Feng Z, Parnes HL and Coltman CA Jr: Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. *J Natl Cancer Inst* 98: 529-534, 2006.
- Berger AP, Deibl M, Strasak A, Bektic J, Pelzer AE, Klocker H, Steiner H, Fritsche G, Bartsch G and Horninger W: Large-scale study of clinical impact of PSA velocity: long-term PSA kinetics as method of differentiating men with from those without prostate cancer. *Urology* 69: 134-138, 2007.
- Loeb S, Roehl KA, Catalona WJ and Nadler RB: Prostate-specific antigen velocity threshold for predicting prostate cancer in young men. *J Urol* 177: 899-902, 2007.
- Mistry K and Cable G: Meta-analysis of prostate-specific antigen and digital rectal examination as screening tests for prostate carcinoma. *J Am Board Fam Pract* 16: 95-101, 2003.
- Draisma G, Boer R, Otto SJ, van dC, I, Damhuis RA, Schroder FH and De Koning HJ: Lead times and overdiagnosis due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 95: 868-878, 2003.
- Vis AN, Hoedemaeker RF, Roobol M, Der Kwast TH and Schroder FH: Tumor characteristics in screening for prostate cancer with and without rectal examination as an initial screening test at low PSA (0.0-3.9 ng/ml). *Prostate* 47: 252-261, 2001.
- Lilja H, Ulmert D and Vickers AJ: Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. *Nat Rev Cancer* 8: 268-278, 2008.
- Loeb S and Catalona WJ: Prostate-specific antigen in clinical practice. *Cancer Lett* 249: 30-39, 2007.
- Schroder FH, Carter HB, Wolters T, van den Bergh RC, Gosselaar C, Bangma CH and Roobol MJ: Early detection of prostate cancer in 2007. Part 1: PSA and PSA kinetics. *Eur Urol* 53: 468-477, 2008.
- Stephan C, Buker N, Cammann H, Meyer HA, Lein M and Jung K: Artificial neural network (ANN) velocity better identifies benign prostatic hyperplasia but not prostate cancer compared with PSA velocity. *BMC Urol* 8: 10, 2008.
- Sardana G, Dowell B and Diamandis EP: Emerging biomarkers for the diagnosis and prognosis of prostate cancer. *Clin Chem* 54: 1951-1960, 2008.
- Herawi M and Epstein JI: Immunohistochemical antibody cocktail staining (p63/HMWCK/AMACR) of ductal adenocarcinoma and Gleason pattern 4 cribriform and noncribriform acinar adenocarcinomas of the prostate. *Am J Surg Pathol* 31: 889-894, 2007.
- Harden SV, Sanderson H, Goodman SN, Partin AA, Walsh PC, Epstein JI and Sidransky D: Quantitative *GSTP1* methylation and the detection of prostate adenocarcinoma in sextant biopsies. *J Natl Cancer Inst* 95: 1634-1637, 2003.
- Reynolds MA, Kastury K, Groskopf J, Schalken JA and Rittenhouse H: Molecular markers for prostate cancer. *Cancer Lett* 249: 5-13, 2007.
- Wright JL and Lange PH: Newer potential biomarkers in prostate cancer. *Rev Urol* 9: 207-213, 2007.
- Laxman B, Morris DS, Yu J, Siddiqui J, Cao J, Mehra R, Lonigro RJ, Tsodikov A, Wei JT, Tomlins SA and Chinnaiyan AM: A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. *Cancer Res* 68: 645-649, 2008.
- Vener T, Derecho C, Baden J, Wang H, Rajpurohit Y, Skelton J, Mehrotra J, Varde S, Chowdary D, Stallings W, Leibovich B, Robin H, Pelzer A, Schafer G, Auprich M, Mannweiler S, Amersdorfer P and Mazumder A: Development of a multiplexed urine assay for prostate cancer diagnosis. *Clin Chem* 54: 874-882, 2008.
- Reynolds MA: Molecular alterations in prostate cancer. *Cancer Lett* 271: 13-24, 2008.

- 25 Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R, Lee C, Montie JE, Shah RB, Pienta KJ, Rubin MA and Chinnaiyan AM: Recurrent fusion of *TMPRSS2* and *ETS* transcription factor genes in prostate cancer. *Science* 310: 644-648, 2005.
- 26 Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B, Yu J, Wang L, Montie JE, Rubin MA, Pienta KJ, Roulston D, Shah RB, Varambally S, Mehra R and Chinnaiyan AM: Distinct classes of chromosomal rearrangements create oncogenic *ETS* gene fusions in prostate cancer. *Nature* 448: 595-599, 2007.
- 27 Demichelis F, Fall K, Perner S, Andren O, Schmidt F, Setlur SR, Hoshida Y, Mosquera JM, Pawitan Y, Lee C, Adami HO, Mucci LA, Kantoff PW, Andersson SO, Chinnaiyan AM, Johansson JE and Rubin MA: *TMPRSS2:ERG* gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene* 26: 4596-4599, 2007.
- 28 Laxman B, Tomlins SA, Mehra R, Morris DS, Wang L, Helgeson BE, Shah RB, Rubin MA, Wei JT and Chinnaiyan AM: Noninvasive detection of *TMPRSS2:ERG* fusion transcripts in the urine of men with prostate cancer. *Neoplasia* 8: 885-888, 2006.
- 29 Morris DS, Tomlins SA, Montie JE and Chinnaiyan AM: The discovery and application of gene fusions in prostate cancer. *BJU Int* 102: 276-282, 2008.
- 30 Stenman UH, Leinonen J, Alfthan H, Rannikko S, Tuhkanen K and 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 Res* 51: 222-226, 1991.
- 31 Lilja H, Christensson A, Dahlen U, Matikainen MT, Nilsson O, Pettersson K and Lovgren T: Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. *Clin Chem* 37: 1618-1625, 1991.
- 32 De Angelis G, Rittenhouse HG, Mikolajczyk SD, Blair SL and Semjonow A: Twenty years of PSA: from prostate antigen to tumor marker. *Rev Urol* 9: 113-123, 2007.
- 33 Stenman UH, Abrahamsson PA, Aus G, Lilja H, Bangma C, Hamdy FC, Boccon-Gibod L and Ekman P: Prognostic value of serum markers for prostate cancer. *Scand J Urol Nephrol Suppl* pp. 64-81, 2005.
- 34 Stephan C, Jung K, Lein M and Diamandis EP: PSA and other tissue kallikreins for prostate cancer detection. *Eur J Cancer* 43: 1918-1926, 2007.
- 35 Catalona WJ, Smith DS, Wolfert RL, Wang TJ, Rittenhouse HG, Ratliff TL and Nadler RB: Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. *JAMA* 274: 1214-1220, 1995.
- 36 Catalona WJ, Partin AW, Slawin KM, Brawer MK, Flanigan RC, Patel A, Richie JP, deKernion JB, Walsh PC, Scardino PT, Lange PH, Subong EN, Parson RE, Gasior GH, Loveland KG and Southwick PC: Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. *JAMA* 279: 1542-1547, 1998.
- 37 Zhang WM, Finne P, Leinonen J, Vesalainen S, Nordling S, Rannikko S and Stenman UH: Characterization and immunological determination of the complex between prostate-specific antigen and alpha2-macroglobulin. *Clin Chem* 44: 2471-2479, 1998.
- 38 Zhang WM, Finne P, Leinonen J, Vesalainen S, Nordling S and Stenman UH: Measurement of the complex between prostate-specific antigen and alpha1-protease inhibitor in serum. *Clin Chem* 45: 814-821, 1999.
- 39 Allard WJ, Zhou Z and Yeung KK: Novel immunoassay for the measurement of complexed prostate-specific antigen in serum. *Clin Chem* 44: 1216-1223, 1998.
- 40 Brawer MK, Meyer GE, Letran JL, Bankson ER, Morris DL, Yeung KK and Allard WJ: Measurement of complexed PSA improves specificity for early detection of prostate cancer. *Urology* 52: 372-378, 1998.
- 41 Lein M, Kwiatkowski M, Semjonow A, Luboldt HJ, Hammerer P, Stephan C, Klevecka V, Taymoorian K, Schnorr D, Recker F, Loening SA and Jung K: A multicenter clinical trial on the use of complexed prostate-specific antigen in low prostate specific antigen concentrations. *J Urol* 170: 1175-1179, 2003.
- 42 Stephan C, Schnorr D, Loening SA and Jung K: Re: Roddam AW, Duffy MJ, Hamdy FC *et al*: Use of prostate-specific antigen (PSA) isoforms for the detection of prostate cancer in men with a PSA level of 2-10 ng/ml: systematic review and meta-analysis. (*Eur Urol* 48: 386-399, 2005). *Eur Urol* 48: 1059-1060, 2005.
- 43 Mikolajczyk SD, Grauer LS, Millar LS, Hill TM, Kumar A, Rittenhouse HG, Wolfert RL and Saedi MS: A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. *Urology* 50: 710-714, 1997.
- 44 Peter J, Unverzagt C, Krogh TN, Vorm O and Hoesel W: Identification of precursor forms of free prostate-specific antigen in serum of prostate cancer patients by immunosorption and mass spectrometry. *Cancer Res* 61: 957-962, 2001.
- 45 Bangma CH, Wildhagen MF, Yurdakul G, Schroder FH and Blijenberg BG: The value of (-7, -5)pro-prostate-specific antigen and human kallikrein-2 as serum markers for grading prostate cancer. *BJU Int* 93: 720-724, 2004.
- 46 Lein M, Semjonow A, Graefen M, Kwiatkowski M, Abramjuk C, Stephan C, Haese A, Chun F, Schnorr D, Loening SA and Jung K: A multicenter clinical trial on the use of (-5, -7) pro prostate specific antigen. *J Urol* 174: 2150-2153, 2005.
- 47 Stephan C, Meyer HA, Paul EM, Kristiansen G, Loening SA, Lein M and Jung K: Serum (-5, -7) proPSA for distinguishing stage and grade of prostate cancer. *Anticancer Res* 27: 1833-1836, 2007.
- 48 Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Amirkhan A, Horninger W, Klocker H and Mikolajczyk SD: Serum proprostate-specific antigen improves cancer detection compared to free and complexed prostate-specific antigen in men with prostate-specific antigen 2 to 4 ng/ml. *J Urol* 170: 2181-2185, 2003.
- 49 Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Horninger W, Klocker H and Mikolajczyk SD: Serum pro-prostate-specific antigen preferentially detects aggressive prostate cancers in men with 2 to 4 ng/ml prostate-specific antigen. *J Urol* 171: 2239-2244, 2004.
- 50 Sokoll LJ, Wang Y, Feng Z, Kagan J, Partin AW, Sanda MG, Thompson IM and Chan DW: (-2)proenzyme prostate-specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study. *J Urol* 180: 539-543, 2008.
- 51 Mikolajczyk SD and Rittenhouse HG: Pro PSA: a more cancer specific form of prostate-specific antigen for the early detection of prostate cancer. *Keio J Med* 52: 86-91, 2003.

- 52 Peyromaure M, Fulla Y, Debre B and Dinh-Xuan AT: Pro PSA : a "pro cancer" form of PSA? *Med Hypotheses* 64: 92-95, 2005.
- 53 Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Wolfert RL, Marks LS, Song W, Wheeler TM and Slawin KM: "BPSA", a specific molecular form of free prostate-specific antigen, is found predominantly in the transition zone of patients with nodular benign prostatic hyperplasia. *Urology* 55: 41-45, 2000.
- 54 Wang TJ, Slawin KM, Rittenhouse HG, Millar LS and Mikolajczyk SD: Benign prostatic hyperplasia-associated prostate-specific antigen (BPSA) shows unique immunoreactivity with anti-PSA monoclonal antibodies. *Eur J Biochem* 267: 4040-4045, 2000.
- 55 Canto EI, Singh H, Shariat SF, Lamb DJ, Mikolajczyk SD, Linton HJ, Rittenhouse HG, Kadmon D, Miles BJ and Slawin KM: Serum BPSA outperforms both total PSA and free PSA as a predictor of prostatic enlargement in men without prostate cancer. *Urology* 63: 905-910, 2004.
- 56 Linton HJ, Marks LS, Millar LS, Knott CL, Rittenhouse HG and Mikolajczyk SD: Benign prostate-specific antigen (BPSA) in serum is increased in benign prostate disease. *Clin Chem* 49: 253-259, 2003.
- 57 Slawin KM, Shariat S and Canto E: BPSA: A Novel Serum Marker for Benign Prostatic Hyperplasia. *Rev Urol 7(Suppl 8): S52-S56*, 2005.
- 58 Stephan C, Cammann H, Lein M, Schrader M, Deger S, Miller K and Jung K: BPHA (bPSA) improves detection of prostate cancer in an artificial neural network. *Urology*: in press 2009.
- 59 de Vries SH, Raaijmakers R, Blijenberg BG, Mikolajczyk SD, Rittenhouse HG and Schroder FH: Additional use of [-2] precursor prostate-specific antigen and "benign" PSA at diagnosis in screen-detected prostate cancer. *Urology* 65: 926-930, 2005.
- 60 Mikolajczyk SD, Marks LS, Partin AW and Rittenhouse HG: Free prostate-specific antigen in serum is becoming more complex. *Urology* 59: 797-802, 2002.
- 61 Nurmikko P, Vaisanen V, Piironen T, Lindgren S, Lilja H and Pettersson K: Production and characterization of novel anti-prostate-specific antigen (PSA) monoclonal antibodies that do not detect internally cleaved Lys145-Lys146 inactive PSA. *Clin Chem* 46: 1610-1618, 2000.
- 62 Nurmikko P, Pettersson K, Piironen T, Hugosson J and 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. *Clin Chem* 47: 1415-1423, 2001.
- 63 Steuber T, Nurmikko P, Haese A, Pettersson K, Graefen M, Hammerer P, Huland H and Lilja H: Discrimination of benign from malignant prostatic disease by selective measurements of single chain, intact free prostate-specific antigen. *J Urol* 168: 1917-1922, 2002.
- 64 Yousef GM and Diamandis EP: The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocr Rev* 22: 184-204, 2001.
- 65 Lundwall A, Band V, Blaber M, Clements JA, Courty Y, Diamandis EP, Fritz H, Lilja H, Malm J, Maltais LJ, Olsson AY, Petraki C, Scorilas A, Sotiropoulou G, Stenman UH, Stephan C, Talieri M and Yousef GM: A comprehensive nomenclature for serine proteases with homology to tissue kallikreins. *Biol Chem* 387: 637-641, 2006.
- 66 Kwiatkowski MK, Recker F, Piironen T, Pettersson K, Otto T, Wernli M and Tscholl R: In prostatism patients the ratio of human glandular kallikrein to free PSA improves the discrimination between prostate cancer and benign hyperplasia within the diagnostic "gray zone" of total PSA 4 to 10 ng/mL. *Urology* 52: 360-365, 1998.
- 67 Partin AW, Catalona WJ, Finlay JA, Darte C, Tindall DJ, Young CY, Klee GG, Chan DW, Rittenhouse HG, Wolfert RL and Woodrum DL: Use of human glandular kallikrein 2 for the detection of prostate cancer: preliminary analysis. *Urology* 54: 839-845, 1999.
- 68 Nakamura T, Scorilas A, Stephan C, Jung K, Soosaipillai AR and Diamandis EP: The usefulness of serum human kallikrein 11 for discriminating between prostate cancer and benign prostatic hyperplasia. *Cancer Res* 63: 6543-6546, 2003.
- 69 Brown DA, Stephan C, Ward RL, Law M, Hunter M, Bauskin AR, Amin J, Jung K, Diamandis EP, Hampton GM, Russell PJ, Giles GG and Breit SN: Measurement of serum levels of macrophage inhibitory cytokine 1 combined with prostate-specific antigen improves prostate cancer diagnosis. *Clin Cancer Res* 12: 89-96, 2006.
- 70 Stephan C, Xu C, Brown DA, Breit SN, Michael A, Nakamura T, Diamandis EP, Meyer H, Cammann H and Jung K: Three new serum markers for prostate cancer detection within a percent free PSA-based artificial neural network. *Prostate* 66: 651-659, 2006.
- 71 Meyer-Siegler KL, Bellino MA and Tannenbaum M: Macrophage migration inhibitory factor evaluation compared with prostate-specific antigen as a biomarker in patients with prostate carcinoma. *Cancer* 94: 1449-1456, 2002.
- 72 Michael A, Stephan C, Kristiansen G, Burckhardt M, Loening SA, Schnorr D and Jung K: Diagnostic validity of macrophage migration inhibitory factor in serum of patients with prostate cancer: a re-evaluation. *Prostate* 62: 34-39, 2005.
- 73 Tahir SA, Ren C, Timme TL, Gdor Y, Hoogveen R, Morrisett JD, Frolov A, Ayala G, Wheeler TM and Thompson TC: Development of an immunoassay for serum caveolin-1: a novel biomarker for prostate cancer. *Clin Cancer Res* 9: 3653-3659, 2003.
- 74 Tahir SA, Frolov A, Hayes TG, Mims MP, Miles BJ, Lerner SP, Wheeler TM, Ayala G, Thompson TC and Kadmon D: Preoperative serum caveolin-1 as a prognostic marker for recurrence in a radical prostatectomy cohort. *Clin Cancer Res* 12: 4872-4875, 2006.
- 75 Paul B, Dhir R, Landsittel D, Hitchens MR and Getzenberg RH: Detection of prostate cancer with a blood-based assay for early prostate cancer antigen. *Cancer Res* 65: 4097-4100, 2005.
- 76 Leman ES, Cannon GW, Trock BJ, Sokoll LJ, Chan DW, Mangold L, Partin AW and Getzenberg RH: EPCA-2: a highly specific serum marker for prostate cancer. *Urology* 69: 714-720, 2007.
- 77 Diamandis EP: POINT: EPCA-2: a promising new serum biomarker for prostatic carcinoma? *Clin Biochem* 40: 1437-1439, 2007.
- 78 Leman ES, Magheli A, Cannon GW, Sokoll LJ, Chan DW, Mangold L, Partin AW and Getzenberg RH: Analysis of a second EPCA-2 epitope as a serum test for prostate cancer. *J Urol (Suppl)* 179: 704, 2008.
- 79 Stattin P, Rinaldi S, Biessy C, Stenman UH, Hallmans G and Kaaks R: High levels of circulating insulin-like growth factor-I increase prostate cancer risk: a prospective study in a population-based nonscreened cohort. *J Clin Oncol* 22: 3104-3112, 2004.

- 80 Finne P, Auvinen A, Koistinen H, Zhang WM, Maattanen L, Rannikko S, Tammela T, Seppala M, Hakama M and Stenman UH: Insulin-like growth factor I is not a useful marker of prostate cancer in men with elevated levels of prostate-specific antigen. *J Clin Endocrinol Metab* 85: 2744-2747, 2000.
- 81 Oliver SE, Holly J, Peters TJ, Donovan J, Persad R, Gillatt D, Pearce A, Hamdy FC, Neal DE and Gunnell D: Measurement of insulin-like growth factor axis does not enhance specificity of PSA-based prostate cancer screening. *Urology* 64: 317-322, 2004.
- 82 Roddam AW, Allen NE, Appleby P, Key TJ, Ferrucci L, Carter HB, Metter EJ, Chen C, Weiss NS, Fitzpatrick A, Hsing AW, Lacey JV Jr, Helzlsouer K, Rinaldi S, Riboli E, Kaaks R, Janssen JA, Wildhagen MF, Schroder FH, Platz EA, Pollak M, Giovannucci E, Schaefer C, Quesenberry CP Jr, Vogelmann JH, Severi G, English DR, Giles GG, Stattin P, Hallmans G, Johansson M, Chan JM, Gann P, Oliver SE, Holly JM, Donovan J, Meyer F, Bairati I and Galan P: Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. *Ann Intern Med* 149: 461-468, 2008.
- 83 von der Kammer H, Jurincic-Winkler C, Horlbeck R, Klippel KF, Pixberg HU and Scheit KH: The potential use of prostatic secretory protein of 94 amino acid residues (PSP94) as a serum marker for prostatic tumor. *Urol Res* 21: 227-233, 1993.
- 84 Nam RK, Reeves JR, Toi A, Dulude H, Trachtenberg J, Emami M, Daigneault L, Panchal C, Sugar L, Jewett MA and Narod SA: A novel serum marker, total prostate secretory protein of 94 amino acids, improves prostate cancer detection and helps identify high grade cancers at diagnosis. *J Urol* 175: 1291-1297, 2006.
- 85 Reeves JR, Dulude H, Panchal C, Daigneault L and Ramnani DM: Prognostic value of prostate secretory protein of 94 amino acids and its binding protein after radical prostatectomy. *Clin Cancer Res* 12: 6018-6022, 2006.
- 86 Wozny W, Schroer K, Schwall GP, Poznanovic S, Stegmann W, Dietz K, Rogatsch H, Schaefer G, Huebl H, Klocker H, Schrattenholz A and Cahill MA: Differential radioactive quantification of protein abundance ratios between benign and malignant prostate tissues: cancer association of annexin A3. *Proteomics* 7: 313-322, 2007.
- 87 Kollermann J, Schlomm T, Bang H, Schwall GP, von Eichel-Streiber C, Simon R, Schostak M, Huland H, Berg W, Sauter G, Klocker H and Schrattenholz A: Expression and prognostic relevance of annexin A3 in prostate cancer. *Eur Urol* 54: 1314-1323, 2008.
- 88 Schostak M, Schwall GP, Poznanovic S, Groebe K, Muller M, Messinger D, Miller K, Krause H, Pelzer A, Horninger W, Klocker H, Hennenlotter J, Feyereabend S, Stenzl A and Schrattenholz A: Annexin A3 in urine: a highly specific noninvasive marker for prostate cancer early detection. *J Urol* 181: 343-353, 2009.
- 89 Hessels D, Klein Gunnewiek JM, van O, I, Karthaus HF, van Leenders GJ, van BB, Kiemeny LA, Witjes JA and Schalken JA: DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 44: 8-15, 2003.
- 90 Groskopf J, Aubin SM, Deras IL, Blase A, Bodrug S, Clark C, Brentano S, Mathis J, Pham J, Meyer T, Cass M, Hodge P, Macairan ML, Marks LS and Rittenhouse H: APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem* 52: 1089-1095, 2006.
- 91 Haese A, de la TA, van PH, Marberger M, Stenzl A, Mulders PF, Huland H, Abbou CC, Remzi M, Tinzi M, Feyereabend S, Stillebroer AB, van Gils MP and Schalken JA: Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *Eur Urol* 54: 1081-1088, 2008.
- 92 Nakanishi H, Groskopf J, Fritsche HA, Bhadkamkar V, Blase A, Kumar SV, Davis JW, Troncoso P, Rittenhouse H and Babaian RJ: PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol* 179: 1804-1809, 2008.
- 93 Whitman EJ, Groskopf J, Ali A, Chen Y, Blase A, Furusato B, Petrovics G, Ibrahim M, Elsamani S, Cullen J, Sesterhenn IA, Brassell S, Rittenhouse H, Srivastava S and McLeod DG: PCA3 score before radical prostatectomy predicts extracapsular extension and tumor volume. *J Urol* 180: 1975-1978, 2008.
- 94 van Gils MP, Hessels D, Hulsbergen-van de Kaa CA, Witjes JA, Jansen CF, Mulders PF, Rittenhouse HG and Schalken JA: Detailed analysis of histopathological parameters in radical prostatectomy specimens and PCA3 urine test results. *Prostate* 68: 1215-1222, 2008.
- 95 Varambally S, Laxman B, Mehra R, Cao Q, Dhanasekaran SM, Tomlins SA, Granger J, Vellaichamy A, Sreekumar A, Yu J, Gu W, Shen R, Ghosh D, Wright LM, Kladney RD, Kuefer R, Rubin MA, Fimmel CJ and Chinnaiyan AM: Golgi protein GOLM1 is a tissue and urine biomarker of prostate cancer. *Neoplasia* 10: 1285-1294, 2008.
- 96 Sreekumar A, Poisson LM, Rajendiran TM, Khan AP, Cao Q, Yu J, Laxman B, Mehra R, Lonigro RJ, Li Y, Nyati MK, Ahsan A, Kalyana-Sundaram S, Han B, Cao X, Byun J, Omenn GS, Ghosh D, Pennathur S, Alexander DC, Berger A, Shuster JR, Wei JT, Varambally S, Beecher C and Chinnaiyan AM: Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 457: 910-914, 2009.
- 97 Jung K: Re: The metabolites citrate, myo-inositol, and spermine are potential age-independent markers of prostate cancer in human expressed prostatic secretions. *European Urology* 54: 1198-1199, 2008.
- 98 Chun FK, Karakiewicz PI, Briganti A, Walz J, Kattan MW, Huland H and Graefen M: A critical appraisal of logistic regression-based nomograms, artificial neural networks, classification and regression-tree models, look-up tables and risk-group stratification models for prostate cancer. *BJU Int* 99: 794-800, 2007.
- 99 Hernandez DJ, Han M, Humphreys EB, Mangold LA, Taneja SS, Childs SJ, Bartsch G and Partin AW: Predicting the outcome of prostate biopsy: comparison of a novel logistic regression-based model, the prostate cancer risk calculator, and prostate-specific antigen level alone. *BJU Int* online available: 2008.
- 100 Shariat SF, Capitanio U, Jeldres C and Karakiewicz PI: Can nomograms be superior to other prediction tools? *BJU Int* online available: 2008.
- 101 Chun FK, Graefen M, Briganti A, Gallina A, Hopp J, Kattan MW, Huland H and Karakiewicz PI: Initial biopsy outcome prediction-head-to-head comparison of a logistic regression-based nomogram *versus* artificial neural network. *Eur Urol* 51: 1236-1240, 2007.
- 102 Finne P, Finne R, Bangma C, Hugosson J, Hakama M, Auvinen A and Stenman UH: Algorithms based on prostate-specific antigen (PSA), free PSA, digital rectal examination and prostate volume reduce false-positive PSA results in prostate cancer screening. *Int J Cancer* 111: 310-315, 2004.

- 103 Kawakami S, Numao N, Okubo Y, Koga F, Yamamoto S, Saito K, Fujii Y, Yonese J, Masuda H, Kihara K and Fukui I: Development, validation, and head-to-head comparison of logistic regression-based nomograms and artificial neural network models predicting prostate cancer on initial extended biopsy. *Eur Urol* 54: 601-611, 2008.
- 104 Djavan B, Remzi M, Zlotta A, Seitz C, Snow P and Marberger M: Novel artificial neural network for early detection of prostate cancer. *J Clin Oncol* 20: 921-929, 2002.
- 105 Finne P, Finne R, Auvinen A, Juusela H, Aro J, Maattanen L, Hakama M, Rannikko S, Tammela TL and Stenman U: Predicting the outcome of prostate biopsy in screen-positive men by a multilayer perceptron network. *Urology* 56: 418-422, 2000.
- 106 Kalra P, Togami J, Bansal BSG, Partin AW, Brawer MK, Babaian RJ, Ross LS and Niederberger CS: A neurocomputational model for prostate carcinoma detection. *Cancer* 98: 1849-1854, 2003.
- 107 Dreiseitl S and Ohno-Machado L: Logistic regression and artificial neural network classification models: a methodology review. *J Biomed Inform* 35: 352-359, 2002.
- 108 Sargent DJ: Comparison of artificial neural networks with other statistical approaches. *Cancer* 91: 1636-1642, 2001.
- 109 Stephan C, Meyer HA, Cammann H, Lein M, Loening SA and Jung K: Re: Chun FKH, Graefen M, Briganti A, Gallina A, HoppJ, Kattan MW, Huland H and Karakiewicz PI. Initial biopsy outcome prediction – head-to-head comparison of a logistic regression-based nomogram *versus* artificial neural network. *Eur Urol* 51: 1236-1243, 2007. *Eur Urol* 51: 1446-1447, 2007.
- 110 Snow PB, Smith DS and Catalona WJ: Artificial neural networks in the diagnosis and prognosis of prostate cancer: a pilot study. *J Urol* 152: 1923-1926, 1994.
- 111 Babaian RJ, Fritsche H, Ayala A, Bhadkamkar V, Johnston DA, Naccarato W and Zhang Z: Performance of a neural network in detecting prostate cancer in the prostate-specific antigen reflex range of 2.5 to 4.0 ng/mL. *Urology* 56: 1000-1006, 2000.
- 112 Stephan C, Jung K, Cammann H, Vogel B, Brux B, Kristiansen G, Rudolph B, Hauptmann S, Lein M, Schnorr D, Sinha P and Loening SA: An artificial neural network considerably improves the diagnostic power of percent free prostate-specific antigen in prostate cancer diagnosis: Results of a 5-year investigation. *Int J Cancer* 99: 466-473, 2002.
- 113 Stephan C, Cammann H, Meyer HA, Lein M and Jung K: PSA and new biomarkers within multivariate models to improve early detection of prostate cancer. *Cancer Lett* 249: 18-29, 2007.
- 114 Schroder F and Kattan MW: The comparability of models for predicting the risk of a positive prostate biopsy with prostate-specific antigen alone: A Systematic Review. *Eur Urol* 54: 274-290, 2008.
- 115 Stephan C, Cammann H, Meyer HA, Muller C, Deger S, Lein M and Jung K: An artificial neural network for five different assay systems of prostate-specific antigen in prostate cancer diagnostics. *BJU Int* 102: 799-805, 2008.
- 116 Stephan C, Xu C, Cammann H, Graefen M, Haese A, Huland H, Semjonow A, Diamandis EP, Remzi M, Djavan B, Wildhagen MF, Blijenberg BG, Finne P, Stenman UH, Jung K and Meyer HA: Assay-specific artificial neural networks for five different PSA assays and populations with PSA 2-10 ng/ml in 4,480 men. *World J Urol* 25: 95-103, 2007.
- 117 Stephan C, Xu C, Finne P, Cammann H, Meyer HA, Lein M, Jung K and Stenman UH: Comparison of two different artificial neural networks for prostate biopsy indication in two different patient populations. *Urology* 70: 596-601, 2007.
- 118 Keller T, Butz H, Lein M, Kwiatkowski M, Semjonow A, Luboldt HJ, Hammerer P, Stephan C and Jung K: Discordance analysis characteristics as a new method to compare the diagnostic accuracy of tests: example of complexed *versus* total prostate-specific antigen. *Clin Chem* 51: 532-539, 2005.
- 119 Stephan C, Meyer HA, Kwiatkowski M, Recker F, Cammann H, Loening SA, Jung K and Lein M: A (-5, -7) ProPSA-based artificial neural network to detect prostate cancer. *Eur Urol* 50: 1014-1020, 2006.
- 120 Stephan C, Kahrs AM, Cammann H, Lein M, Schrader M, Deger S, Miller K and Jung K: A (-2)proPSA-based artificial neural network significantly improves differentiation between prostate cancer and benign prostatic diseases. *Prostate* 69: 198-207, 2009.
- 121 Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, Scardino PT, Hugosson J and Lilja H: A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. *BMC Med* 6: 19, 2008.
- 122 Stephan C, Jung K, Soosaipillai A, Yousef GM, Cammann H, Meyer H, Xu C and Diamandis EP: Clinical utility of human glandular kallikrein 2 within a neural network for prostate cancer detection. *BJU Int* 96: 521-527, 2005.
- 123 Stephan C, Meyer HA, Cammann H, Nakamura T, Diamandis EP and Jung K: Improved prostate cancer detection with a human kallikrein 11 and percentage free PSA-based artificial neural network. *Biol Chem* 387: 801-805, 2006.
- 124 Emami N and Diamandis EP: Utility of kallikrein-related peptidases (KLKs) as cancer biomarkers. *Clin Chem* 54: 1600-1607, 2008.
- 125 Parekh DJ, Ankerst DP, Troyer D, Srivastava S and Thompson IM: Biomarkers for prostate cancer detection. *J Urol* 178: 2252-2259, 2007.
- 126 Scorilas A, Plebani M, Mazza S, Basso D, Soosaipillai AR, Katsaros N, Pagano F and Diamandis EP: Serum human glandular kallikrein (hK2) and insulin-like growth factor 1 (IGF-1) improve the discrimination between prostate cancer and benign prostatic hyperplasia in combination with total and % free PSA. *Prostate* 54: 220-229, 2003.
- 127 Zhigang Z, Jieming L, Su L and Wenlu S: Serum insulin-like growth factor I/free prostate specific antigen (IGF-I/fPSA) ratio enhances prostate cancer detection in men with total PSA 4.0-10.0 ng/ml. *J Surg Oncol* 96: 54-61, 2007.
- 128 Aubin SM, Miick S, Williamsen S, Hodge P, Meinke J, Blase A, Hessels D, Schalken J, Rittenhouse H and Groskopf J: Improved prediction of prostate biopsy outcome using PCA3, *TMPRSS2:ERG* gene fusion and serum PSA. *J Urol Suppl* 179: 725, 2008.
- 129 Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB and Schalken JA: Detection of *TMPRSS2-ERG* fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. *Clin Cancer Res* 13: 5103-5108, 2007.

Received January 5, 2009

Revised March 13, 2009

Accepted April 2, 2009