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Review

Prostate-specific antigen and other serum and urine markers in prostate cancer

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ABSTRACT

Prostate-specific antigen (PSA) is one of the most widely used tumor markers, and strongly correlates with the risk of harboring from prostate cancer (PCa). This risk is visible already several years in advance but PSA has severe limitations for PCa detection with its low specificity and low negative predictive value. There is an urgent need for new biomarkers especially to detect clinically significant and aggressive PCa. From all PSA-based markers, the FDA-approved Prostate Health Index (phi) shows improved specificity over percent free and total PSA. Other serum kallikreins or sarcosine in serum or urine show more diverging data. In urine, the FDA-approved prostate cancer gene 3 (PCA3) has also proven its utility in the detection and management of early PCa. However, some aspects on its correlation with aggressiveness and the low sensitivity at very high values have to be re-examined. The detection of a fusion of the androgen regulated TMPRSS2 gene with the ERG oncogene (from the ETS family), which acts as transcription factor gene, in tissue of ~50% of all PCa patients was one milestone in PCa research. When combining the urinary assays for TMPRSS2:ERG and PCA3, an improved accuracy for PCa detection is visible. PCA3 and phi as the best available PCa biomarkers show an equal performance in direct comparisons.

Contents

Abbreviations: %fPSA, ratio of fPSA to tPSA (in percent) also known as f/tPSA ratio or percent free PSA; %[−2]proPSA, percentage of [-2]proPSA to fPSA; A2M, alpha2-macroglobulin; ACT, alpha-1-antichymotrypsin; ANN, artificial neural network; API, alpha1-protease inhibitor; AUC, area under the receiver-operating characteristic curve; BPH, benign prostate hyperplasia; bPSA, "benign"PSA a clipped subform of free PSA; cPSA, sum of the complexed PSA with ACT and API; DRE, digital rectal examination; ERSPC, European Randomized Study of Screening for Prostate Cancer; fPSA: free, unbound PSA to other proteins; iPSA, "intact" or unclipped free PSA form; KLK1, pancreatic/renal kallikrein; KLK2, human glandular kallikrein 2; KLK3, identical with PSA; KLK4 to KLK15, all other kallikreins 4 to 15; LR, logistic regression; MIC-1, macrophage inhibitory cytokine 1; MIF, cytokine macrophage migration inhibitory factor; OR, odds ratio; PCa, prostate cancer; PCA3 prostate cancer gene 3, noncoding messenger RNA; phi, prostate health index; proPSA, a free PSA subform containing a seven amino acid N-terminal pro-leader peptide in its native form that is termed [−7]proPSA which is rapidly truncated by proteolytic cleavage to other proPSA subforms [−4]proPSA, [−5]proPSA or [−2]proPSA; PSA: prostate-specific antigen, a widely used serum marker for prostate cancer management; PSA-A2M, PSA complexed with A2M; PSA-ACT, PSA bound to ACT; PSA-API, PSA bound to alpha1-protease inhibitor; ROC, receiver-operating characteristic; tPSA, total PSA; TMPRSS2, androgen regulated gene that is fused with the ERG transcription factor gene.

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1. PSA and prostate cancer

1.1. Prostate cancer incidence and mortality

The increasing use of serum prostate-specific antigen (PSA) within the last 25 years lifted prostate cancer (PCa) to the most frequent neoplasia in men in the Western world [1,2]. In Europe, an incidence of 416 700 new cases and a mortality of 92 200 cancer deaths per year was reported for 2012 [3]. There have been an estimated 233 000 new cases and 29 480 cancer deaths in the USA for 2014 [1]. Worldwide approximately 900 000 new cases and 258 000 PCa related deaths have been reported for 2008 [4]. For the year 2030, an incidence of 1.7 million PCa and an annual mortality rate of 0.5 million men have been proposed [4].

1.2. Biology of PSA and its correlation with PCa

PSA is one of the most widely used biomarkers. The serine protease PSA was detected in serum in 1980 [5]. Since then it has revolutionized the management of PCa [6]. Biologically, PSA is responsible for semen liquefaction and secreted into the seminal plasma [7]. Usually, the retrograde release of PSA into the bloodstream is a rare event in healthy men. It occurs with a frequency of less than one PSA molecule per million secreted PSA molecules, leading to a concentration of <4 ng/ml in serum, which is a million-fold lower than the PSA concentration in seminal plasma (0.5–5 mg/ml). Only a destruction of the basement membrane of prostate epithelial cells may result in excessive escape of PSA into the blood circulation [8].

However, besides PCa, also benign prostate diseases, as well as physical trauma of the prostate can result in significant increases of serum PSA. Thus, elevated serum PSA levels only indicate pathologies of the prostate gland, including PCa, but PSA is not cancer-specific. In the USA, almost 55% of all men aged 40 years or older were estimated to have a PSA test during the preceding 2 years [9]. There is a strong correlation between the serum PSA concentration and the risk of PCa [10–12]. In 2950 American men undergoing prostate biopsy and showing PSA values <4 ng/ml and a 7 year follow-up the prevalence of PCa increased from 6.6% among men with a PSA of 0–0.5 ng/ml to 10.1% and 17% for PSA values of 0.6–1.0 and 1.1–2 ng/ml and finally to 23.9% and 26.9% among those with PSA values of 2.1–3 and 3.1–4 ng/ml [11]. In the way of this landmark trial, clinicians began to consider PSA as a continuum, with increasing levels reflecting greater risk. The concept of a low PSA limit where below PCa could not be detected was largely abandoned. Further large studies show similar results. In 5855 screened Swedish men, the PCa detection rates during a 7.6 year follow-up were for the following PSA ranges: 0–0.49 ng/ml, 0% (0 from 958); 0.5–0.99 ng/ml, 0.9% (17/1992); 1–1.49 ng/ml, 4.7% (54/1138); 1.5–1.99 ng/ml, 12.3% (70/571); 2–2.49 ng/ml, 21.4% (67/313); 2.5–2.99 ng/ml, 25.2% (56/222); 3–3.99 ng/ml, 33.3% (89/267); 4–6.99 ng/ml, 38.9% (103/265); 7–9.99 ng/ml, 50% (30/60); >10 ng/ml, 76.8% (53/69) [10]. However, regular biopsy was only offered at PSA levels >3 ng/ml [10]. Noteworthy, within 3 years there was no single case of a detected PCa in those men with an initial PSA level of <1 ng/ml [10]. Comparable PCa detection rates were obtained from 10 523 screened Dutch men [12], where biopsy was performed at PSA concentrations >4 ng/ml or if digital rectal examination (DRE) and/or transrectal ultrasound were suspicious for cancer. Here, the PCa detection rates for the respective PSA ranges were as follows: PSA 0–0.9 ng/ml, 0.2% (9/3858); 1–1.9 ng/ml, 1.3% (43/3305); 2–2.9 ng/ml, 2.2% (29/1314); 3–3.9 ng/ml, 6.3% (46/734); 4–9.9 ng/ml, 21.7% (238/1095) and >10 ng/ml, 52.1% with 113 PCa from 217 screened men [12]. Another study in 26 111 screened men with 2122 detected PCa had increasing PCa detection rates as follows: PSA 0–1 ng/ml, 1%, PSA 1.1–2.5 ng/ml: 8%; PSA 2.6–4 ng/ml: 20%; PSA 4.1–10: 31% and PSA >10 ng/ml: 56% [13]. Furthermore, in those 1356 men who underwent radical prostatectomy, the organ confined tumors decreased in the 5 PSA-ranges from 82% and 78% over 77% to 67% and 44% showing also a relationship between PSA and aggressiveness and tumor stage [13]. Those large studies clearly prove the relationship between increasing PSA values and an increasing risk of harboring a PCa, as reviewed [14–16]. There is strong evidence that PSA can be used to predict the PCa risk many years in advance [14].

Another important point is the risk of metastatic PCa and its relationship with PSA measurement. Data from 76 813 men from 4 centers of the European Randomized Study of Screening for Prostate Cancer (ERSPC) were evaluated for the presence of metastatic disease by imaging or by PSA values >100 ng/ml at diagnosis or during 12 years of follow-up. Overall, 666 men with metastatic PCa were detected, 256 in the screening arm and 410 in the control arm. The cumulative incidence of metastatic PCa was significantly reduced with PSA screening ($p < 0.001$) and the relative reduction was 30% ($p = 0.001$) in the intention-to-screen analysis or already 42% ($p = 0.0001$) for those men who were actually screened [17].

Furthermore, recent long-term follow-up data for 25–30 years show that also the risk to die from metastatic PCa with 44% is strongly increased for men aged 45 to 55 years when their PSA is within the 10th percentile (≥1.6 or ≥2.4 ng/ml) [18]. Those men with PSA values below the respective medians (<0.68 or <0.85 ng/ml) only have a risk to die of metastatic PCa of lower than 0.3% [18].

So far, it seems unlikely that PSA can be replaced as first line screening parameter within the next years. PSA is also the key parameter for prediction [17], staging and monitoring of PCa [15]. PSA improves treatment selection and patient care, and predicts the risk of complications and disease recurrence [14].

1.3. PSA limitations

However, for PCa detection but not for monitoring or prediction, PSA has incisive limitations. As already mentioned, benign prostate diseases such as benign prostate hyperplasia (BPH) or prostatitis as well as manipulations (bicycling, digital rectal examination, catheterization) of the prostate can also cause elevated PSA serum concentrations [6,19]. This leads to a low specificity and low positive predictive value if a single PSA measurement is used to predict PCa, especially in the

PSA "gray zone" of 2–10 ng/ml [6,20]. The large biological variations in the measured PSA concentrations with up to 20–30% is a further drawback [21]. A simple repeat measurement of PSA can avoid significant numbers of unnecessary prostate biopsies [22]. But approximately 60–80% of all biopsies indicate no PCa at the pathology report and are therefore unnecessary.

1.4. PSA derivatives

To increase the specificity of PSA, different parameters have been developed in the beginning of the 1990s like PSA velocity (change of PSA over a time period) including the PSA doubling time, PSA density (ratio of PSA to prostate volume) or age-/race-specific reference ranges. Results have been summarized [23–26] but all these PSA based parameters have been only partially successful and will be therefore not be described in detail. There is still an urgent need to improve specificity of PSA.

2. PSA based serum markers

2.1. PSA complexes with proteinase inhibitors

In the early 1990s two independent groups found PSA to exist in different molecular forms [27,28]. Approximately 65–95% of PSA is bound to alpha-1-antichymotrypsin (PSA–ACT) while the remaining PSA circulates as free (unbound) PSA (fPSA). PSA–ACT is higher in PCa patients compared with non-PCa patients [28]. The reason for this difference between BPH and PCa patients is not completely understood. It is assumed that the loss of tissue architecture in PCa causes a quicker access of active PSA from the prostate epithelium into the circulation. Then, the protease inhibitor ACT and other inhibitors like alpha1-protease inhibitor (API) or alpha2-macroglobulin (A2M) can complex with PSA more easily [29]. If active PSA reaches the circulation from normal or BPH cells, it first leaks backwards into the extracellular space, where it is susceptible to proteolytic degradation [29]. After degradation, PSA becomes inactive and can form complexes only to a very small degree with ACT but still with A2M [30]. Therefore, in patients without PCa the amount of PSA–ACT is lower and subsequently the relative amount of fPSA is higher. The different amounts of PSA–ACT and fPSA are generated extracellular or in the blood circulation. This hypothesis is confirmed because PSA in prostatic tissue is present almost exclusively as uncomplexed, free PSA [31–33].

PSA complexes with API or A2M became measurable in the late 1990s [34,35]. PSA–API [36] and PSA–A2M [37] are significantly higher in BPH patients. While the amount of PSA–API in BPH and PCa patients was 1.6% and 0.9% of PSA [36], the PSA–A2M amounted to 12% and 8% of PSA, respectively [37]. The higher amount of inactive PSA after its backward leakage in BPH patients may lead to more PSA–A2M complexes since inactive PSA can still bind to A2M [38]. However, the measurement for PSA–A2M needs rather complicated methods [35] and the very small amounts of API to total PSA (tPSA) are analytically challenging [36] so that both assays never became commercially available.

The sum of PSA–ACT and PSA–API is also directly measurable as complex PSA (cPSA) [39]. The cPSA can only reach comparable results to the ratio of fPSA to tPSA (f/tPSA ratio or percent free PSA, %fPSA) when also used as ratio to tPSA [40,41] despite a meta-analysis stated that %fPSA and cPSA show comparable results [42]. From the point of theoretical view, PSA–ACT and cPSA are the most cancer specific forms of PSA. Likewise, %fPSA is theoretically inferior to PSA–ACT, but measurement of %fPSA is metrologically more favorable and has therefore become the preferred method [43]. No study has shown an advantage of the PSA–ACT or the PSA–ACT/tPSA ratio, compared with %fPSA, to enhance the specificity of PCa detection (cited in [44]). Therefore, the vast majority of clinical utility studies on molecular forms of PSA have been published on %fPSA.

2.2. Clinical relevance of %fPSA

Since the middle of the 1990s the %fPSA has become clinically relevant to improve specificity of PSA alone [45–49]. This has been confirmed for the 4–10 ng/ml tPSA range (reviewed in [50]). But also for lower PSA values <4 ng/ml, %fPSA has shown its clinical value [51–53]. A meta-analysis on %fPSA found an area under the ROC curve (AUC) of 0.68 for more than 2800 patients within the tPSA "gray zone" of 4–10 ng/ml [54]. But the authors concluded that %fPSA can only be a useful adjunct to PSA-based screening when reaching extreme values such as <7% [54]. With used %fPSA cutoffs at 90–95% sensitivity (which is equal to absolute %fPSA cutoff values of ~15%–25% depending on the used assays), the number of unnecessary biopsies could be reduced by approximately 10–20%. This has been shown for the indications of initial and repeat biopsies. For a correct interpretation, influencing factors of %fPSA should be considered. With increasing prostate volume as one of the clinical signs of BPH, %fPSA also increases (cited in [44]). Notably, a larger prostate volume in PCa patients is correlated with higher %fPSA. PCa patients with large prostate glands have higher %fPSA values due to the coexistence of BPH and PCa. Therefore, %fPSA cannot distinguish between BPH and PCa in those patients and should be used preferentially in patients with a smaller gland volume <40 ml [55]. Other relationships are more divergent like a negative correlation with tPSA, staging and grading PCa with %fPSA or relationships with age or prostatic intraepithelial neoplasia (cited in [44]).

It has been proposed by Stephan et al. [56] in 1997 and later by Lee et al. [57] to use %fPSA as a priority decision tool for first time biopsy. Data from more than 10 000 screened men, where 758 underwent prostate biopsy, confirm the impact of %fPSA on the PCa probability [58]. For instance, at a tPSA value of 4 ng/ml, the PCa probability increases from 3% to 39% when the %fPSA decreases from 35% to 7% [58]. Even when the tPSA is 10 ng/ml, the PCa probability is below 15% when %fPSA is above 25% [58]. For low PSA concentrations <3 ng/ml, %fPSA has also shown its value in 17 680 screened Finnish men with a median follow-up of almost 6 years and 327 detected PCa [59]. The risk of PCa detection is 33% when the tPSA is only 2–3 ng/ml but %fPSA is below 10% [59]. Furthermore, men with a %fPSA in the lowest quartile (<14.2%) had an almost 7-fold higher PCa risk compared with those with a %fPSA level in the highest quartile (>23.7%) [59]. Recent data from a large Danish study with 27 179 men in a case–control study that subsequently identified 874 men with PCa found a 10-fold higher PCa incidence when %fPSA is below 15% compared with %fPSA values >20% after a 14-year follow-up [60]. In men older than 57 years this incidence is already 18-fold higher for %fPSA <15% compared with >20% [60]. Another research group more cautiously argued, that %fPSA might be less useful as a long-term predictor of PCa [61]. In a retrospective study in more than 21 000 men of ages 44–50, the archived samples of 462 PCa were compared with 1222 matched controls after 25 years [61]. A multivariable kallikrein-model incorporating tPSA, fPSA, %fPSA, and the human glandular kallikrein 2 (KLK2) had no better predictive power than tPSA [61]. Later studies with the same kallikrein panel in other cohorts found better results for %fPSA within the panel [62,63]. However, this was for prediction on biopsy results, where %fPSA improved the AUC and not for long-term prognosis.

Although the long-term value of %fPSA is debatable, this ratio has been established especially within multivariable models such as artificial neural networks (ANN) or logistic regression (LR) to predict the PCa risk (reviewed in [64]). Table 1 (modified from reference [64]) shows the improvement of %fPSA and ANN or LR models compared with tPSA published between 1998 and 2004 [65–72]. Regardless of the different used tPSA and fPSA assays and the different PSA ranges investigated, enhanced specificities by using %fPSA within ANN or LR models were observed [73]. However, only some models are available in the internet [67,73,74]. Exemplarily, in Figs. 1 and 2 the respective models by Finne et al. [67] and Stephan et al. [73] are shown.

Table 1
Examples for multivariable models using %fPSA for diagnosis of PCa (1998–2004).

Authors [Ref.] (n of pts.; % with PCa)	Year	Screening	Model (ranking)	PSA assays (company)	tPSA range (ng/ml)	Contributing factors (if numbered, then by value)	AUC	Specificity at 95% sensitivity
Carlson et al. [66] (n = 3773; 33% PCa)	1998	No	LR	Tosoh (Dianon)	4–20	1. %fPSA 2. Age 3. tPSA	n.a.	34 (LR) 23 (%fPSA)
Virtanen et al. [72] (n = 212; 25% PCa)	1999	Yes	1. LR 2. ANN	ProStatus (Wallac)	3–10 (3–45)	1. %fPSA 2. DRE 3. Heredity	0.81 (LR for tPSA 3–45) %fPSA n.a.	n.a.
Finne et al. [67] (n = 656; 23% PCa)	2000	Yes	1. ANN 2. LR	ProStatus (Wallac)	4–10	1. %fPSA 2. Volume 3. DRE 4. tPSA	n.a.	33 (ANN) 24 (LR) 19 (%fPSA)
Babaian et al. [65] (n = 151; 25% PCa)	2000	Yes	ANN	Tandem R (Beckman Coulter)	2.5–4	%fPSA, tPSA, age, PAP, CK	0.74 ANN (0.64%fPSA)	51 (ANN) 39 (PSAD) 10 (%fPSA)
Horninger et al. [69] (n = 3474; n.a.)	2001	Yes	ANN LR	Abbot IMX (Abbott)	n.a. PSA > 4 or DRE+	Age, tPSA, %fPSA, DRE, volume, PSAD, PSAD-TZ, TZ-volume	n.a.	~27 (ANN) ~13 (%fPSA) ~13 (tPSA)
Stephan et al. [71] (n = 1188; 61% PCa)	2002	No	ANN LR	IMMULITE (Bayer)	2–20	1. DRE 2. %fPSA 3. Volume 4. tPSA 5. Age	0.86 (ANN) 0.75 (%fPSA)	43 (ANN) 26 (%fPSA)
Remzi et al. [70] (n = 820; 10% PCa)	2003	No	ANN, LR	AxSYM (Abbott)	4–10	tPSA, %fPSA, volume, PSAD, PSAD-TZ, TZ-volume	0.83 (ANN) 0.79 (LR) 0.745 (%fPSA)	68 (ANN) 54 (LR) 33.5 (%fPSA)
Finne et al. [68] (n = 1775; 22% PCa)	2004	Yes	1. LR 2. ANN	ProStatus (Wallac)	4–10	1. DRE 2. %fPSA 3. Volume 4. tPSA	0.764 (LR) 0.760 (ANN) 0.718 (%fPSA)	22 (LR) 19 (ANN) 17 (%fPSA)

Abbreviations: AUC: area under the (ROC) curve; n.a.: not available; LR: logistic regression; ANN: artificial neural network, PAP: prostate alkaline phosphatase, CK: creatin kinase; PSAD: PSA density, PSAD-TZ: transition zone density; DRE: digital rectal examination.

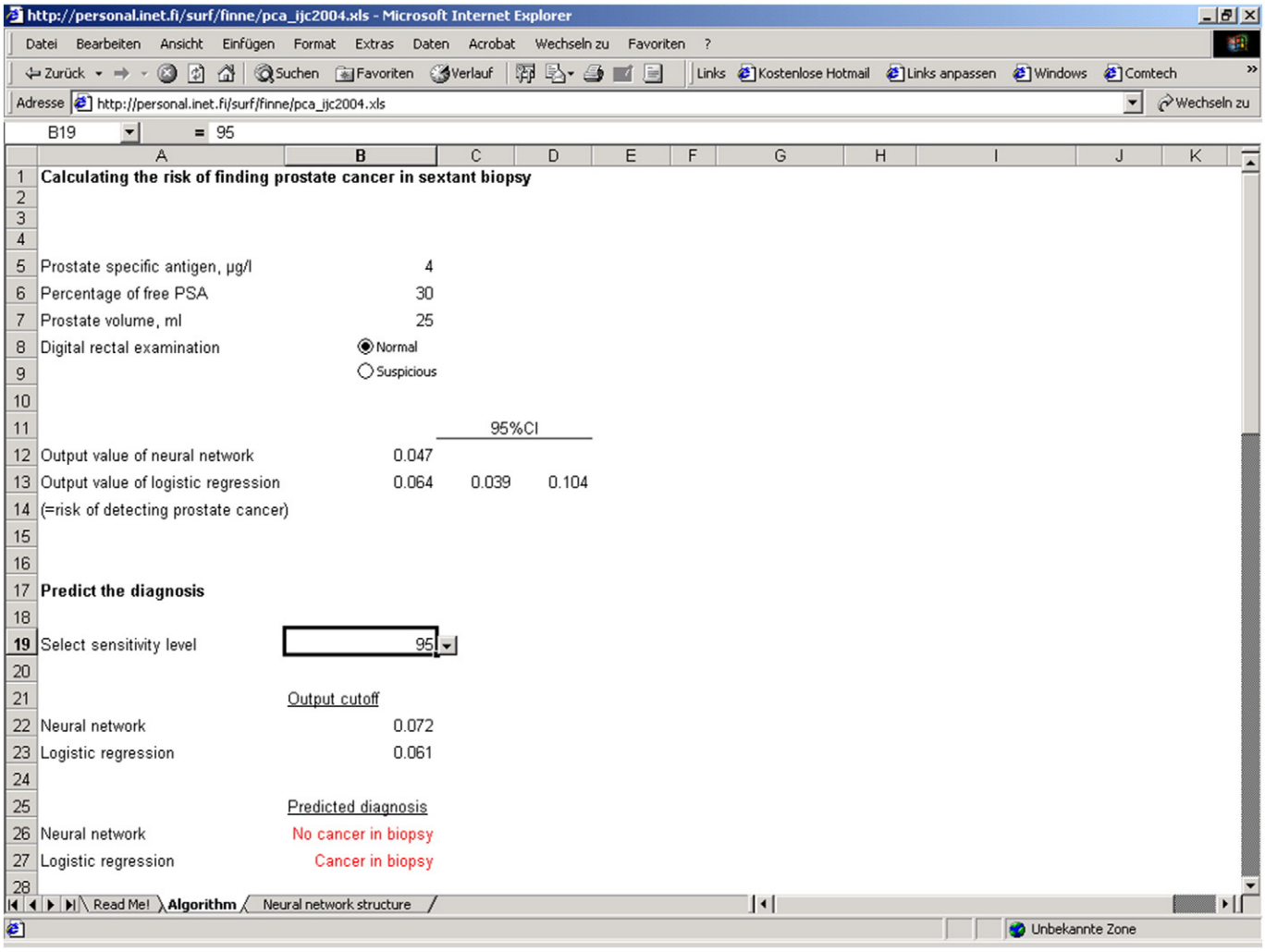

Fig. 1. The program at www.finne.info to estimate the risk of PCa based on ANN and LR at the 95% sensitivity level.

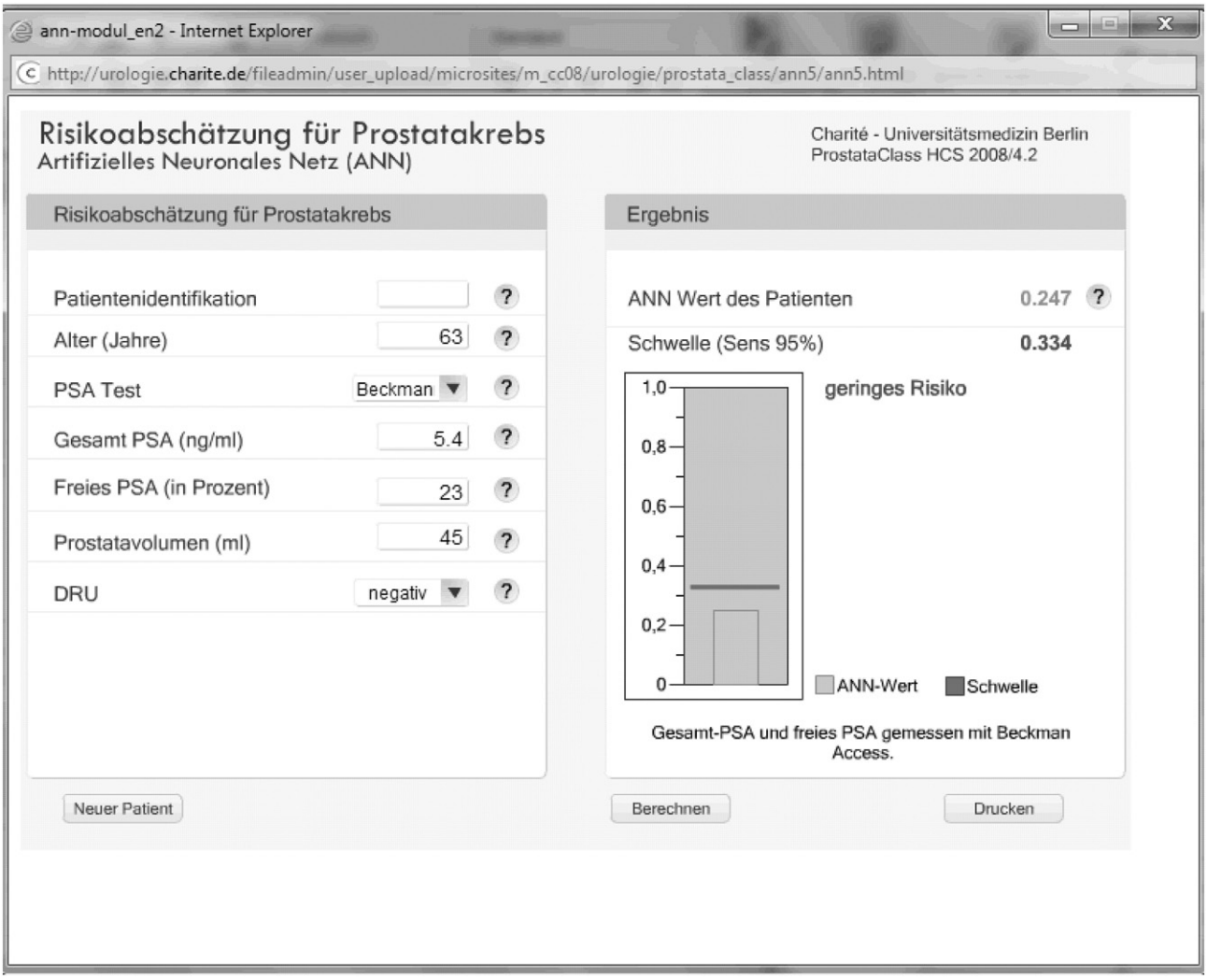

Fig. 2. Program "ProstataClass" version 2008 for 5 different PSA assays at http://urologie.charite.de and the link: "ProstataClass". Example of the ANN output (only available in German) indicating "geringes Risiko" (lower risk)" at the 95% sensitivity level. The German input values are Alter [english: age], PSA Test [PSA assay], Gesamt PSA [total PSA], Freies PSA (in Prozent) [percent free PSA], Prostatavolumen [prostate volume] and DRU [digital rectal exam: DRE].

2.3. Subforms of free PSA

With the beginning of the new millennium researchers focused to determine subforms of fPSA [32,75–79]. The free PSA became more complex [80]. Fig. 3 indicates the different molecular forms of PSA.

The so called "benign" PSA (bPSA) is a clipped subform of free PSA that is highly associated with the transition zone of the prostate, containing BPH nodules [32]. The bPSA could be used as marker for BPH, but was unable to distinguish between BPH and PCa [81,82]. However, within a multivariable model, bPSA could improve the specificity of %fPSA by approximately 15% [82].

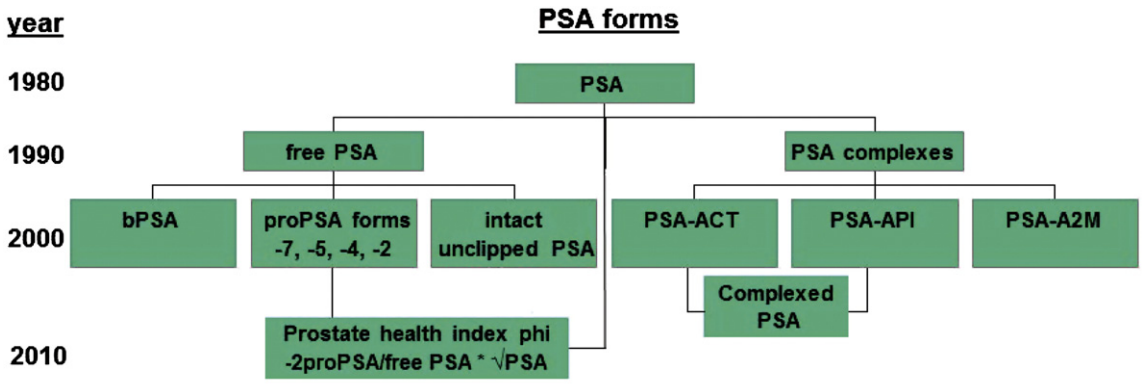

Fig. 3. Molecular forms of PSA and the Prostate Health Index phi including the respective times of detection.

Another fPSA subform was detected by using anti-PSA antibodies that do not recognize internally cleaved PSA at Lys145–Lys146. This special PSA subform was termed "intact", unclipped PSA (iPSA) [77]. Although iPSA could distinguish between PCa and BPH [78,83], its further use has been limited since a commercial assay is lacking.

The third subform, proPSA contains a seven amino acid N-terminal pro-leader peptide in its native form and is termed [−7]proPSA. The cleavage of [−7]proPSA naturally occurs rapidly to form enzymatically active PSA. However, the [−7]proPSA is also rapidly truncated by proteolytic cleavage to [−4]proPSA, [−5]proPSA or [−2]proPSA. [-2] proPSA is not cleaved further and it does accumulate in cancer tissue.

A research assay measuring the [−7, -5]proPSA was of limited usefulness and had subsequently not been commercialized (reviewed in [44]. Also, the [-4]proPSA was not specific enough for PCa detection [84].

However, only the [−2]proPSA [84,85] and especially the commercial and FDA-approved [−2]proPSA [86–89] showed the expected further improvement in specificity over %fPSA. A specific formula using [−2]proPSA, fPSA and tPSA was empirically found to be best to discriminate between PCa and non-PCa. Since 2010 the Prostate Health Index phi (calculated as: [−2]proPSA/fPSA × √PSA) has been used [90, 91]. These data on phi have been confirmed in large multicenter cohorts and it further seems that phi may preferentially detect aggressive PCa [86,89,92,93].

2.4. Clinical importance of Prostate Health Index phi

In 2012, [−2]proPSA was approved by the FDA to be used for initial biopsy decision in men with PSA in the range of 4–10 ng/ml and negative DRE. A comprehensive review summarizes all aspects on different proPSA forms including the clinical utility of [−2]proPSA and phi as well as the cost-effectiveness of phi [94]. The addition of phi to the common screening strategy with PSA alone slightly increases the costs of the blood tests but could reduce the number of required office visits, laboratory tests and prostatic biopsies [94]. Incorporating phi into a ERSPC screening model resulted in a predicted reduction in negative biopsies by 29%, a reduction in detected PCa by 2% and a total cost reduction for PCa care by 2% [95]. The summarized health economic studies suggest the addition of phi to PSA-based screening alone [94].

A recent meta-analysis for phi and the percentage of [−2]proPSA to fPSA (%[-2]proPSA) analyzed data from more than 5000 biopsied men within the 2–10 ng/ml tPSA range [96]. Filella and Gimenez [96] described at 90% sensitivity a pooled specificity of approximately 32% for phi and %[−2]proPSA and found both parameters to be superior to tPSA and %fPSA in detecting PCa.

Increasing phi values were associated with an increased probability of detecting Gleason ≥7 PCa [86,89]. Two studies in more than 2200 men independently found that the relative risk of any PCa is 3.6-fold [89] to 4.7-fold [86] higher in those men with phi values the highest as compared with the lowest quartile. The risk of a Gleason ≥ 7 PCa increases 1.6-fold with phi values the within highest quartile [86]. In the other study, phi had significantly higher median values for patients with a Gleason ≥ 7 PCa compared with a Gleason score < 7 PCa and the proportion of Gleason ≥ 7 PCa increased with the phi score [89]. In a prospective study in 566 men by Sokoll et al. [93], the authors found %[-2]proPSA to be increased with increasing Gleason score ($p < 0.001$) and higher in aggressive cancers ($p = 0.03$). A prospective evaluation in 350 consecutive men who underwent radical prostatectomy found significantly higher %[-2]proPSA and phi levels in patients with pT3 disease, pathologic Gleason sum ≥ 7, and Gleason sum upgrading [97]. Thus, preoperative determination of %[-2]proPSA and phi appear to predict patients with more aggressive disease on final pathology and might therefore be useful in the preoperative counseling of patients [94]. If phi preferentially detects aggressive PCa, it might become an increasing role for PCa patients under active surveillance [94].

It seems that the earlier specificity enhancement of %fPSA compared with tPSA in studies between 1995 and 2002 now disappears and that only phi shows currently a real specificity improvement over tPSA and also over %fPSA. The reasons for the decreased %fPSA performance are unclear. The harmonization of almost all PSA assays to WHO standardized tPSA and fPSA assays with approximately 20% lower values might be one reason.

Interestingly, the further improvement of %fPSA by using it within multivariable models (see table 1) could now not be repeated when using phi within multivariable models. The AUC-gain was very modest or not visible [89,98]. The reason for that phenomenon is also unclear. From the mathematical view the formula for phi seems to be near the optimum when using tPSA, fPSA and [−2]proPSA. As reviewed by Roobol et al. [99], the inclusion of new biomarkers such as urinary prostate cancer gene 3 (PCA3) and [-2]proPSA in risk calculators amounted only to a marginal improvement in the accuracy of these prediction tools. Despite this, phi showed overall promising data since 2010, especially when focusing to detect aggressive PCa so that phi could fill the gap of those urgently needed marker for detecting PCa [100].

3. Other kallikreins beside PSA (KLK3)

3.1. The kallikrein family

Until the end of the last millennium, only three human kallikrein genes were known: the pancreatic/renal kallikrein KLK1, KLK2 and KLK3, which is widely known as PSA [101]. Until 2001, 12 new members of the human kallikrein family have been characterized so that this family of proteases has now 15 members [102]. To simplify the classification of all kallikreins (human, mouse, rat), a new nomenclature has been introduced in 2006 [103].

The human kallikrein genes are named *KLK1* to *KLK15* and they encode for the proteins KLK1 to KLK15. All of them share several significant homologies and all are located on human chromosome 19q13.4. All kallikrein genes are highly homologous at the DNA and amino acid levels (40–80%). Many kallikreins are regulated by steroid hormones [102].

3.2. The human glandular kallikrein 2 (KLK2)

KLK2 has been investigated extensively and in 1997 three different groups found that KLK2 can convert proPSA to active PSA [104–106]. *KLK2* and *PSA* share the highest homology among all kallikreins with 78% and 80% identity at the amino acid and DNA level, respectively [102]. Thus, it was not astonishing that KLK2 could add significant information for detecting PCa, especially at lower PSA values [107–111]. KLK2 was initially found to discriminate between high and low grade as well as between pT2 and pT3 PCa [112–114], but others could not confirm these data [115,116]. Also, within multivariable models, the usefulness of KLK2 remained limited [117,118]. In congruence to PSA, KLK2 has also been found to exist in different molecular forms such as free KLK2, ACT-KLK2, complexed KLK2, proKLK2 or others (reviewed in [44] and [50]). All findings on KLK2 subforms have not been transferred into commercial assays or into clinical routine use.

3.3. Other kallikreins (beside KLK2 and PSA)

Beside KLK2 and PSA, at least 6 other kallikreins (*KLK4*, *KLK10-13* and *KLK15*) are also expressed in relatively high amounts in prostate tissue [102]. Like KLK2, KLK4 also has been found to activate proPSA to PSA [119]. While immunoassays for KLK4, KLK12 and KLK13 are not available, data on KLK11 have been promising [120,121] but were not independently confirmed. Two groups published KLK10 immunoassays [122,123], but data on PCa are lacking. KLK15 has been shown to be highly overexpressed in prostate tissue [124]. KLK15 has prognostic

value [125,126] and a subsequent immunoassay has been developed [127] but similar to KLK11, an independent confirmation is still lacking. Detailed reviews on the role of all kallikreins in cancer management have been published within the last years [102,128] and recently [129].

Currently only one working group continues to publish on the use of multiple kallikreins such as tPSA, fPSA, "intact" PSA and KLK2 to further improve the PCa management [62,63]. Improvements in AUC by 0.08 [63] or 0.1 to 0.15 [62] by using the panel in comparison to base-models with at least PSA and age and partially DRE status show promising results. It has been speculated that there might be a potential synergistic association when using this four kallikrein panel together with the currently best serum parameter phi, which itself is already a combination of three PSA forms [94].

But again, commercial assays for "intact" PSA, KLK2 or KLK11 are not available and therefore a widespread use in the future seems unlikely.

4. Other prostate cancer serum marker

Details on several markers like caveolin, IGF, PSP94 that have never reached clinical significance or at least assay commercialization have been already reviewed [130]. It is known, that publications on EPCA-2 had to be withdrawn, as earlier already criticized by Prof. Diamandis [131]. Other markers will be named below.

4.1. MIC-1, MIF, S100A8/9, Spondin-2 and Galectin-3

Briefly, the macrophage inhibitory cytokine 1 (MIC-1) reduced in a diagnostic algorithm together with tPSA and %fPSA the rate of potentially unnecessary biopsies by 27% and was an independent predictor of Gleason ≥ 7 PCa in a study in 1000 men [132]. In 1442 PCa patients with a mean follow-up of almost 5 years the MIC-1 serum levels independently predicted poor cancer-specific survival with an almost 3-fold higher cancer death rate in patients with serum levels in the highest quartile compared with men with serum levels in the lowest quartile [133].

The cytokine macrophage migration inhibitory factor (MIF) was initially elevated in PCa patients compared with non-PCa patients in totally 509 analyzed patients but histological diagnosis was available only in 152 men [134]. Others could not confirm these data while MIF values were in fact decreased and not significantly higher in PCa patients [135]. Furthermore, combined data on MIC-1, MIF and KLK11 revealed lower AUCs of 0.6, 0.56 and 0.6 for those three markers than for tPSA and %fPSA showing their limited value to discriminate PCa from BPH [136].

The calcium-binding proteins S100A8 and S100A9 have been proposed as novel diagnostic markers for PCa [137]. S100A9 serum levels were significantly elevated in PCa patients compared with BPH patients or healthy individuals [137]. Another group could not confirm these data by using the same assays and showed lower diagnostic performance for S100A8 and S100A9 than for %fPSA [138]. While PSA rapidly decreases after radical prostatectomy, the S100 proteins showed an increase in their concentrations after surgery [138]. Therefore it is unlikely that S100 proteins can be used as diagnostic or prognostic PCa markers.

Two serum markers, the extracellular matrix protein Spondin-2 [139], and Galectin-3, a tumor-associated protein [140], have been published in 2013. Spondin-2 showed an extremely high AUC of 0.95 as compared with %fPSA (0.81), sarcosine (0.67) and tPSA (0.56) in serum samples from 286 PCa and 68 non-PCa patients. The greater accuracy for Spondin-2 was also present in a subset of 88 patients with PSA values of <4 ng/ml [139]. This confirmed the initial data for Spondin-2 with an AUC of 0.94 but there were only 13 healthy controls [141]. The galectin-3 levels were in contrast only compared in the sera of metastatic PCa patients with non-cancer patients [140]. However, independent confirmation of these data for both markers is still lacking. There is usually a long way off and novel markers such as these must add clinical value to the existing standards before they are adopted into practice [142].

4.2. Sarcosine in serum

As already mentioned in the study on Spondin-2 [139], where also sarcosine in serum has been evaluated but with limited success, another group published more promising data on sarcosine [143]. In serum samples from 1122 PCa cases (813 non-aggressive and 309 aggressive) and 1112 controls sarcosine was measured by using high-throughput liquid chromatography–mass spectrometry [143]. The authors found an increased PCa risk with increasing sarcosine levels with an odds ratio (OR) for the highest quartile versus the lowest quartile of 1.30, (95% confidence interval (CI) 1.02–1.65; $p = 0.03$). When stratified by disease aggressiveness, the OR was 1.44 (95% CI 1.11–1.88; $p = 0.006$). Thus, elevated serum sarcosine levels were here associated with an increased PCa risk [143]. In contrast, a nested case–control study among 3000 PCa cases and 3000 controls taken from a Norwegian prospective cohort of 317 000 men with baseline serum samples, found high sarcosine and glycine concentrations to be associated with a reduced PCa risk of borderline significance (sarcosine: highest vs. lowest quintile OR: 0.86, CI 0.72–1.01; $p = 0.03$; glycine: OR 0.83, CI 0.70–1.00; $p = 0.07$) [144]. However, one group could not prove any sarcosine differences in 57 serum samples of healthy controls, PCa and metastatic PCa patients [145] but others found sarcosine to be significantly higher in metastatic PCa as compared with non-metastatic PCa patients [146]. Another study showed a superiority of %fPSA as compared with sarcosine for tPSA levels of 4–10 ng/ml and >10 ng/ml [147]. However, in those 145 patients with tPSA levels <4 ng/ml, sarcosine had a higher predictive value than tPSA and %fPSA and it was further positively associated with the percentage of low/intermediate-grade cancers [147]. Others did not find differences regarding plasma levels of sarcosine between early and advanced PCa stages [148].

Taken together, the contradictory results of all the above-mentioned studies on sarcosine in serum or plasma show inconsistent data [139,143–148].

5. Urine markers

5.1. Sarcosine

In 2009, a widely recognized paper has been speculated on a potential role of sarcosine as a derivative of the amino acid glycine for PCa detection and prognosis [149]. In approximately 100 patients the authors found sarcosine to be significantly higher in urine sediments and supernatants in men with PCa as compared with men without biopsy proven PCa [149]. The AUC for sarcosine was 0.69 for those 53 patients within the PSA gray zone 2–10 ng/ml while PSA showed a significant lower AUC of 0.53 [149]. When using all available patients, sarcosine had AUCs of 0.71 and 0.67. It should be noted that the mean PSA values in this study were not equally distributed between PCa patients (mean: 11.4 ng/ml, range: 2.7–111 ng/ml) and biopsy negative men (mean: 5.3 ng/ml, range: 1.1–10 ng/ml) [149].

Contrarily, another study in 139 men with more comparable PSA values between biopsy proven PCa (median 7.07 ng/ml, range: 1.7–19.2 ng/ml) and non-PCa patients (median 6.05 ng/ml, range: 1–17.7 ng/ml) found significantly lower sarcosine values in PCa patients (806 μmol/mol) compared with non-PCa patients (929 μmol/mol) and no difference between healthy men (788 μmol/mol) and PCa patients [150]. Also, %fPSA had a significantly larger AUC than sarcosine (0.81 vs. 0.63; $p = 0.012$), and PSA was equal to sarcosine (0.64 vs. 0.63; $p = 0.93$) [150]. In this study, sarcosine was measured by gas chromatography–mass spectrometry using a commercial amino acid assay and sarcosine values were normalized to urine creatinine [150]. There was a strong correlation ($r_s = 0.86$) between the sarcosine and creatinine

concentrations showing that the occurrence of sarcosine in urine is due to renal excretion [150]. Sarcosine is a naturally occurring metabolite and not specific to prostate tissue nor is its tissue concentration related to tumor aggressiveness or recurrence [151]. This is in contrast to PCA3, which is specific to the prostate and especially PCa but not found elsewhere [152]. Therefore it is unlikely that sarcosine is suitable as a marker for PCa detection. In addition, it was demonstrated that DRE did not significantly change urinary sarcosine levels in PCa patients [150] in contrast to PCA3, where DRE affects the PCA3 score [153].

Further details on sarcosine including its biochemical cycle, analytical methods for quantification and different pathways of the connection of synthesis of sarcosine and PCa development have been reviewed very recently [154].

5.2. PCA3

PCA3 is a noncoding messenger RNA (mRNA) that is 66-fold overexpressed in PCa tissue as measured by quantitative real time-PCR [155]. Early in house urine assays showed already promising data when using the ratio of PSA mRNA to PCA3 mRNA (reviewed in [156]).

In 2006, an automated assay for PCA3 was introduced [157]. That assay was later named ProgensaTM PCA3. This assay was 2012 FDA-approved for use in conjunction with other patient information to aid in the decision for repeat prostate biopsy in men ≥50 years. In 2008, this test was validated with producing correct values for variability and reproducibility in a multicenter study [153], where urine collection after prostate massage pressing three times each lobe was considered as ideal. In the same year, two independent prospective multicenter studies with more than 1000 patients together confirmed the excellent clinical value of the PCA3 assay [158,159]. Haese et al. [158] found that PCA3 was independent of prostate volume, patient's age, as well as tPSA and that this marker was also more effective than %fPSA. While the authors concluded that the probability of a positive repeat biopsy increases with the PCA3 score, they also emphasized that a low PCA3 score does not exclude the existence of a PCa [158]. Further, the PCa detection rate was only 47% in those patients with PCA3 scores >100 [158]. This problem has been reported in several other studies (reviewed in [156]) proving the low sensitivity with a PCA3 cutoff of 100. It shows that there might be an existence of tumors that are not diagnosed on biopsy or repeat biopsy [160] but there is still no clear explanation due to tissue specificity of PCA3 and proven differential expression of PCA3 in normal and cancerous prostate cells.

Nonetheless, several studies have proven the clinical value of PCA3 to improve specificity over PSA and mostly also over %fPSA to detect PCa (reviewed in [156,161–164]). To simplify the overview, only studies with at least 200 biopsy proven patients have been summarized in Table 2 [158,159,165–184]. In the table, data were provided on detected percentage of PCa, ROC analysis and the used PCA3 cutoff. The AUC values for PCA3 are between 0.64 and 0.76 while most studies show AUCs around 0.7 with the exception of the data by two Italian groups [176,185]. Scattoni et al. [185] found an AUC of only 0.59 for PCA3 and Perdona et al. [176] described a high AUC of 0.83. However, the used cutoffs offer a large range from 10 to 51. One of the largest study on PCA3 so far was conducted at 50 urology practices, evaluating finally 1913 men with PSA higher than 2.5 ng/ml and/or abnormal DRE in a community-based environment [186]. In this study, the better performance of PCA3 (AUC 0.706) in comparison with PSA (AUC 0.569) was confirmed and the authors reported that PCA3 in conjunction with PSA increases the AUC to 0.72 [186]. According to their data, the traditional PCA3 cutoff of 35 reduced the number of negative biopsies from 1089 to 248, but false negatives (missed cancers) increased significantly from 17 to 413 [186]. Better results were obtained lowering the PCA3 cutoff to 10, reducing the number of false positives to 35.4% (385 patients). The percentage of false-negative results only increased to 5.6% (108 patients), and the majority of these PCa were less aggressive with a Gleason score of 6 [186].

While the improved specificity over PSA is continuously evident in most studies, there is insufficient evidence that PCA3 testing leads to improved health outcomes [161]. Due to the lack of appropriate cut-off level with acceptable performance characteristics, the PCA3 test is not capable to replace PSA a first-line test in clinical practice [162]. However, addition of PCA3 to risk assessment tools leads to an increase in predictive capability [162].

The first nomogram that included PCA3 led to a 5% gain in the predictive accuracy (AUC from 0.68 to 0.73, $p = 0.04$) when analyzing data on more than 800 biopsied men [172]. Another nomogram only for initial biopsy patients in almost 700 patients showed similarly an increased accuracy by 4.5–7.1% related to PCA3 inclusion [174]. Surprisingly, a comparison of two PCA3-based risk calculators revealed a higher AUC for PCA3 as single parameter (0.83) in comparison to both models

Table 2
Selected studies with more than 200 subjects on PCA3 (2007–2013).

Authors [Ref.] (n of pts.; % of PCa)	Year	PCA3 cutoff	AUC	Sensitivity	Specificity
Marks et al. [168] (n = 226; 27% PCa)	2007	35	0.68	58	72
Haese et al. [158] (n = 463; 28% PCa)	2008	35	0.66	47	72
Deras et al. [159] (n = 570; 36% PCa)	2008	35	0.69	54	74
Ankerst et al. [169] (n = 443; 28% PCa)	2008	25	0.665	63	60
Chun et al. [172] (n = 809; 39% PCa)	2009	17	0.68	81	45
Hessels et al. [167] (n = 336; 40% PCa)	2010	35	0.72	61	74
Auprich et al. [166] (n = 621; 41% PCa)	2010	17 (24, 35)	0.73–0.75	88	45
Roobol et al. [177] (n = 721; 17% PCa)	2010	35	0.635	68	56
Ploussard et al. [181] (n = 301; 24% PCa)	2010	35 (25, 30)	0.69	44–59	67–79
Aubin et al. [182] (n = 1072; 18% PCa)	2010	35	0.69	48	79
De la Taille et al. [183] (n = 516; 40% PCa)	2011	35	0.76	64	76
Perdona et al. [176] (n = 218; 33.5% PCa)	2011	51	0.83	70	81
Bollito et al. [171] (n = 1237; 26% PCa)	2012	35 (39, 50)	0.68	73 (n = 949 at PSA 4–10)	49 (n = 949 at PSA 4–10)
Crawford et al. [186] (n = 1913; 42% PCa)	2012	10 (25, 35)	0.71	86.5	37
Stephan et al. [178] (n = 246; 45% PCa)	2013	28	0.74	73	64
Hansen et al. [174] (n = 692; 46% PCa)	2013	21	0.74	79	59
Ochiai et al. [175] (n = 633; 42% PCa)	2013	35	0.74	66.5	72
Scattoni et al. [185] (n = 211; 33% PCa)	2013	16.5 (13.5, 23.5)	0.59	80 (90)	16–34
Tombal et al. [179] (n = 1024; 18% PCa)	2013	20	n.a.	87	55
Gittelman et al. [173] (n = 466; 22% PCa)	2013	25	0.71	77.5	57
Ferro et al. [165] (n = 300; 36% PCa)	2013	22	0.73	90	40
Goode et al. [180] (n = 456; 19% PCa)	2013	35	0.73	62	75
Ruffion et al. [184] (n = 601; 46% PCa)	2013	35	0.74	63	72

Abbreviations: AUC: area under the (ROC) curve; n.a.: not available.

(AUC: 0.796 and 0.715) but the number of PCa patients was only 73 [176].

Different to PSA, the PCA3 value is not influenced by several factors such as prostate volume [158], prostatitis [187] or medication with 5-alpha-reductase inhibitors [170], which are advantages for a more unrestricted use of this tumor marker compared with PSA or %fPSA or partially also phi. Regardless of some limitation of PCA3 with its complicated measurement procedure, relative high costs (~300 Euros), the unresolved low sensitivity at very high values >100 or the unresolved relationships with tumor volume and aggressiveness (unpublished data of a multicenter study in cystoprostatectomy patients), this urinary biomarker has shown its clinical value since more than 5 years (see Table 2). When using PCA3 together with the best clinical judgment, the excellent diagnostic accuracy is outstanding with a negative predictive value of 99% for clinical relevant PCa with Gleason ≥ 7 [179]. New combinations together with multiparametric MRI open new fields for PCA3 to be useful in PCa diagnosis [188]. Further information on all clinical aspects of PCA3 including the relationship to cancer aggressiveness and subsequent use of PCA3 for active surveillance have been reviewed recently [156].

5.3. PCA3 comparison with phi

One important aspect on tumor markers to decide on the clinical validity is their direct comparison. Both FDA-approved markers, serum [−2]proPSA including phi and urinary PCA3 have been compared only by 3 different groups [98,178,185]. One group published already 3 times on this comparison with initially 151 patients [98], followed by 160 patients [189], and now with 300 patients [165]. The study in 300 men only with first time biopsy did not find a difference between phi (AUC 0.77) and PCA3 (AUC 0.73) for the tPSA range 2–10 ng/ml [165]. One group reported a borderline significance for a better performance of phi (AUC 0.7) as compared with PCA3 (AUC 0.59; $p = 0.043$) [185]. This significance diminished when separating the patients undergoing initial ($n = 116$) or repeat ($n = 95$) biopsy but PCA3 did not increase the predictive accuracy in a multivariable model while phi did so [185]. There was a first study to compare phi, PCA3 and the urinary assay for the gene fusion TMPRSS2:ERG [178]. PCA3 showed overall and in all subgroups the largest AUCs between 0.7 and 0.77 but the differences to phi (AUCs 0.68 to 0.71) were never significant [178]. On the other hand, only phi was able to distinguish between Gleason < 7 and ≥ 7 PCa ($p = 0.0005$) while PCA3 failed ($p = 0.8$). However, the gain in predictive accuracy was moderate in a model with both markers [178].

5.4. TMPRSS-2

There is compelling evidence to consider genomic rearrangements as an initial event in oncogenesis (cited in [190]). Major advances have been made in detecting genomic alterations in PCa [191]. The detection of gene alterations involving the androgen regulated TMPRSS2 gene and the ETS transcription factor genes in PCa patients was one milestone in prostate cancer research within the last 10 years [192]. Approximately 50% of all PCa patients do have the TMPRSS2 fusion with the ETS family member that is regarded as key PCa oncogene [192–194]. TMPRSS2:ERG fusion in PCa is the most common genetic aberration described to date in human solid tumors. Based on these important findings in PCa tissue, a urinary assay using the same platform like PCA3 has been developed [195]. TMPRSS2:ERG in PCa tissue and in urine showed a strong correlation demonstrating a high tumor specificity of this marker [196]. In 2011 a high AUC of 0.77 was reported for the TMPRSS2:ERG urinary assay in a small cohort with only 15 PCa patients, which was higher than the AUC for PCA3 with 0.65 [197]. So far, only one study reported separate data on the TMPRSS2:ERG urine assay in a larger ($n = 246$) and more balanced cohort [178]. ROC data on TMPRSS2:ERG in urine have been somewhat disappointing with a significant lower AUC (0.63) than for PCA3 (0.74) but with no difference to phi (AUC 0.68) [178]. All other studies only reported AUCs when using PCA3 and TMPRSS2:ERG together so that no separate evaluation of TMPRSS2:ERG was possible [198].

Further, the use of TMPRSS2:ERG and PCA3 in widely used PCa risk calculators has been published [198–200]. TMPRSS2:ERG had independent additional predictive value to PCA3 and the ERSPC risk calculator parameters for predicting PCa [199]. TMPRSS2:ERG had prognostic value, whereas PCA3 did not [199]. Urinary TMPRSS2:ERG was associated with indicators of clinically significant PCa, including tumor volume, high Gleason score at prostatectomy, and upgrading of Gleason grade at prostatectomy [200]. TMPRSS2:ERG, in combination with PCA3, improved the performance of the multivariate Prostate Cancer Prevention Trial risk calculator in predicting cancer on biopsy [200]. Another model using additional urinary markers beside TMPRSS2:ERG and PCA3 found an AUC of 0.89 [201].

However, there is a clear rational basis for the need of combining PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis [202]. The PCA3 expression typically increased from BPH to normal prostate tissue (3 times) and PCa tissue (30 times) while the TMPRSS2:ERG gene fusion expression was found in 8.3% of the BPH, 15.6% of the normal prostate tissue and 50% of the PCa samples [202]. These findings are in congruence with the earlier described TMPRSS2:ERG gene fusion expression in approximately 50% of PCa patients [192–194]. The mentioned study showed that most of the false-negative results of the PCA3 test were corrected by TMPRSS2:ERG [202]. The combination of both markers was capable to improve the sensitivity for PCa diagnosis [202].

To summarize the available data on potential urinary PCa biomarkers, there are several new markers usually measured by one research group only [203–205]. Preanalytical conditions more difficult than in serum and urine collection are subject to variability, which may result in conflicting clinical results [163]. Detecting prostate cancer in urine is technically feasible but few markers have been validated in multiple large sample sets [163]. Details on further urine marker have been published elsewhere [163,206–208]. However, advanced clinical studies have identified only PCA3 and TMPRSS2:ERG fusion transcripts as promising RNA markers for cancer detection and prognosis [163].

6. Conclusion

Within the next years, PSA will continue to be a first line biomarker for PCa management. PSA's predictive value several years before the cancer is visible and the strong correlation with the PCa risk is remarkable. This is an advantage for PSA as compared with the urine marker PCA3. But the low specificity of PSA forces the need for new markers. Here, the [-2]proPSA-based phi correlates with aggressive cancer and shows improved specificities over %fPSA and PSA, while other markers only provide ambiguous data. PCA3 has proven its utility in studies with more than 18 000 men but the questionable correlation with tumor aggressiveness has to be re-examined. Comparisons between phi and PCA3 as the best available PCa biomarkers so far show an equal performance of both parameters. Multivariable programs and the combined urinary assays for TMPRSS2:ERG and PCA3 have potential to improve accuracy for PCa detection.

References

[1] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, 2014, CA Cancer J. Clin. 64 (2014) 9–29.
[2] J. Ferlay, H.R. Shin, F. Bray, D. Forman, C. Mathers, D.M. Parkin, Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, Int. J. Cancer 127 (2010) 2893–2917.
[3] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W. Coebergh, H. Comber, D. Forman, F. Bray, Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer 49 (2013) 1374–1403.

[4] M.M. Center, A. Jemal, J. Lortet-Tieulent, E. Ward, J. Ferlay, O. Brawley, F. Bray, International variation in prostate cancer incidence and mortality rates, Eur. Urol. 61 (2012) 1079–1092.
[5] L.D. Papsidero, M.C. Wang, L.A. Valenzuela, G.P. Murphy, T.M. Chu, A prostate antigen in sera of prostatic cancer patients, Cancer Res. 40 (1980) 2428–2432.
[6] T.J. Polascik, J.E. Oesterling, A.W. Partin, Prostate specific antigen: a decade of discovery—what we have learned and where we are going, J. Urol. 162 (1999) 293–306.
[7] H. Lilja, J. Oldbring, G. Rannevik, C.B. Laurell, Seminal vesicle-secreted proteins and their reactions during gelation and liquefaction of human semen, J. Clin. Invest. 80 (1987) 281–285.
[8] U.H. Stenman, Prostate-specific antigen, clinical use and staging: an overview, Br. J. Urol. 79 (Suppl. 1) (1997) 53–60.
[9] E. Hughes, G. Kilmer, Y. Li, B. Valluru, J. Brown, G. Colclough, S. Geathers, H. Roberts, L. Elam-Evans, L. Balluz, Surveillance for certain health behaviors among states and selected local areas — United States, 2008, MMWR Surveill. Summ. 59 (2010) 1–221.
[10] G. Aus, J.E. Damber, A. Khatami, H. Lilja, J. Stranne, J. Hugosson, Individualized screening interval for prostate cancer based on prostate-specific antigen level: results of a prospective, randomized, population-based study, Arch. Intern. Med. 165 (2005) 1857–1861.
[11] I.M. Thompson, D.K. Pauler, P.J. Goodman, C.M. Tangen, M.S. Lucia, H.L. Parnes, L.M. Minasian, L.G. Ford, S.M. Lippman, E.D. Crawford, J.J. Crowley, C.A. Coltman Jr., Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter, N. Engl. J. Med. 350 (2004) 2239–2246.
[12] F.H. Schröder, I. van der Cruijsen-Koeter, H.J. De Koning, A.N. Vis, R.F. Hoedemaker, R. Kranse, Prostate cancer detection at low prostate specific antigen, J. Urol. 163 (2000) 806–812.
[13] J.A. Antenor, M. Han, K.A. Roehl, R.B. Nadler, W.J. Catalona, Relationship between initial prostate specific antigen level and subsequent prostate cancer detection in a longitudinal screening study, J. Urol. 172 (2004) 90–93.
[14] G. Botchorishvili, M.P. Matikainen, H. Lilja, Early prostate-specific antigen changes and the diagnosis and prognosis of prostate cancer, Curr. Opin. Urol. 19 (2009) 221–226.
[15] H. Lilja, D. Ulmert, A.J. Vickers, Prostate-specific antigen and prostate cancer: prediction, detection and monitoring, Nat. Rev. Cancer 8 (2008) 268–278.
[16] S. Loeb, H.B. Carter, W.J. Catalona, J.W. Moul, F.H. Schroder, Baseline prostate-specific antigen testing at a young age, Eur. Urol. 61 (2012) 1–7.
[17] F.H. Schroder, J. Hugosson, S. Carlsson, T. Tammela, L. Maattanen, A. Auvinen, M. Kwiatkowski, F. Recker, M.J. Roobol, Screening for prostate cancer decreases the risk of developing metastatic disease: findings from the European Randomized Study of Screening for Prostate Cancer (ERSPC), Eur. Urol. 62 (2012) 745–752.
[18] A.J. Vickers, D. Ulmert, D.D. Sjoberg, C.J. Bennette, T. Bjork, A. Gerdtsson, J. Manjer, P.M. Nilsson, A. Dahlin, A. Bjartell, P.T. Scardino, H. Lilja, Strategy for detection of prostate cancer based on relation between prostate specific antigen at age 40–55 and long term risk of metastasis: case–control study, BMJ 346 (2013) f2023.
[19] T.A. Stamey, M. Caldwell, J.E. McNeal, R. Nolley, M. Hemenez, J. Downs, The prostate specific antigen era in the United States is over for prostate cancer: what happened in the last 20 years? J. Urol. 172 (2004) 1297–1301.
[20] K. Mistry, G. Cable, Meta-analysis of prostate-specific antigen and digital rectal examination as screening tests for prostate carcinoma, J. Am. Board Fam. Pract. 16 (2003) 95–101.
[21] G. Soletormos, A. Semjonow, P.E. Sibley, R. Lamerz, P.H. Petersen, W. Albrecht, P. Bialk, M. Gion, F. Junker, H.P. Schmid, H. Van Poppel, Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice, Clin. Chem. 51 (2005) 1342–1351.
[22] R. Singh, D. Cahill, R. Popert, T.S. O'Brien, Repeating the measurement of prostate-specific antigen in symptomatic men can avoid unnecessary prostatic biopsy, BJU Int. 92 (2003) 932–935.
[23] J. Hernandez, I.M. Thompson, Prostate-specific antigen: a review of the validation of the most commonly used cancer biomarker, Cancer 101 (2004) 894–904.
[24] S. Loeb, W.J. Catalona, Prostate-specific antigen in clinical practice, Cancer Lett. 249 (2007) 30–39.
[25] R.G. Nixon, M.K. Brawer, Enhancing the specificity of prostate-specific antigen (PSA): an overview of PSA density, velocity and age-specific reference ranges, Br. J. Urol. 79 (Suppl. 1) (1997) 61–67.
[26] A.J. Vickers, C. Savage, M.F. O'Brien, H. Lilja, Systematic review of pretreatment prostate-specific antigen velocity and doubling time as predictors for prostate cancer, J. Clin. Oncol. 27 (2009) 398–403.
[27] H. Lilja, A. Christensson, U. Dahlen, M.T. Matikainen, O. Nilsson, K. Pettersson, T. Lovgren, Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin, Clin. Chem. 37 (1991) 1618–1625.
[28] U.H. Stenman, J. Leinonen, H. Alfthan, S. Rannikko, K. Tuhkanen, O. Alfthan, 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 (1991) 222–226.
[29] U.H. Stenman, J. Leinonen, W.M. Zhang, P. Finne, Prostate-specific antigen, Semin. Cancer Biol. 9 (1999) 83–93.
[30] J. Leinonen, W.M. Zhang, U.H. Stenman, Complex formation between PSA isoenzymes and protease inhibitors, J. Urol. 155 (1996) 1099–1103.
[31] K. Jung, B. Brux, M. Lein, B. Rudolph, G. Kristiansen, S. Hauptmann, D. Schnorr, S.A. Loening, P. Sinha, Molecular forms of prostate-specific antigen in malignant and benign prostatic tissue: biochemical and diagnostic implications, Clin. Chem. 46 (2000) 47–54.
[32] S.D. Mikolajczyk, L.S. Millar, T.J. Wang, H.G. Rittenhouse, R.L. Wolfert, L.S. Marks, W. Song, T.M. Wheeler, K.M. Slawin, "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 (2000) 41–45.
[33] D.K. Ornstein, C. Englert, J.W. Gillespie, C.P. Paweletz, W.M. Linehan, M.R. Emmert-Buck, E.F. Petricoin III, Characterization of intracellular prostate-specific antigen from laser capture microdissected benign and malignant prostatic epithelium, Clin. Cancer Res. 6 (2000) 353–356.
[34] W.M. Zhang, J. Leinonen, N. Kalkkinen, U.-H. Stenman, Prostate-specific antigen forms a complex with and cleaves a_1-protease inhibitor in vitro, Prostate 33 (1997) 87–96.
[35] W.M. Zhang, P. Finne, J. Leinonen, S. Vesalainen, S. Nordling, S. Rannikko, U.H. Stenman, Characterization and immunological determination of the complex between prostate-specific antigen and alpha2-macroglobulin, Clin. Chem. 44 (1998) 2471–2479.
[36] W.M. Zhang, P. Finne, J. Leinonen, S. Vesalainen, S. Nordling, U.H. Stenman, Measurement of the complex between prostate-specific antigen and alpha1-protease inhibitor in serum, Clin. Chem. 45 (1999) 814–821.
[37] W.M. Zhang, P. Finne, J. Leinonen, J. Salo, U.H. Stenman, Determination of prostate-specific antigen complexed to alpha(2)-macroglobulin in serum increases the specificity of free to total PSA for prostate cancer, Urology 56 (2000) 267–272.
[38] U.H. Stenman, P. Finne, W.M. Zhang, J. Leinonen, Prostate-specific antigen and other prostate cancer markers, Urology 56 (2000) 893–898.
[39] W.J. Allard, Z. Zhou, K.K. Yeung, Novel immunoassay for the measurement of complexed prostate-specific antigen in serum, Clin. Chem. 44 (1998) 1216–1223.
[40] M. Lein, M. Kwiatkowski, A. Semjonow, H.J. Luboldt, P. Hammerer, C. Stephan, V. Klevecka, K. Taymoorian, D. Schnorr, F. Recker, S.A. Loening, K. Jung, A multicenter clinical trial on the use of complexed prostate specific antigen in low prostate specific antigen concentrations, J. Urol. 170 (2003) 1175–1179.
[41] C. Stephan, D. Schnorr, S.A. Loening, K. Jung, 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 2005;48:386–99, Eur. Urol. 48 (2005) 1059–1060.
[42] A.W. Roddam, M.J. Duffy, F.C. Hamdy, A.M. Ward, J. Patnick, C.P. Price, J. Rimmer, C. Sturgeon, P. White, N.E. Allen, 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 (2005) 386–399.
[43] U.H. Stenman, Detection of complexes between prostate-specific antigen and protease inhibitors in plasma, Clin. Chem. 56 (2010) 1895–1896.
[44] C. Stephan, K. Jung, M. Lein, E.P. Diamandis, PSA and other tissue kallikreins for prostate cancer detection, Eur. J. Cancer 43 (2007) 1918–1926.
[45] W.J. Catalona, D.S. Smith, R.L. Wolfert, T.J. Wang, H.G. Rittenhouse, T.L. Ratliff, R.B. Nadler, Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening, JAMA 274 (1995) 1214–1220.
[46] W.J. Catalona, A.W. Partin, K.M. Slawin, M.K. Brawer, R.C. Flanigan, A. Patel, J.P. Richie, J.B. deKernion, P.C. Walsh, P.T. Scardino, P.H. Lange, E.N. Subong, R.E. Parson, G.H. Gasior, K.G. Loveland, P.C. Southwick, 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 (1998) 1542–1547.
[47] K. Jung, C. Stephan, M. Lein, W. Henke, D. Schnorr, B. Brux, P. Schürenkämper, S.A. Loening, Analytical performance and clinical validity of two free prostate-specific antigen assays compared, Clin. Chem. 42 (1996) 1026–1033.
[48] A.W. Partin, W.J. Catalona, P.C. Southwick, E.N. Subong, G.H. Gasior, D.W. Chan, Analysis of percent free prostate-specific antigen (PSA) for prostate cancer detection: influence of total PSA, prostate volume, and age, Urology 48 (1996) 55–61.
[49] C. Stephan, K. Jung, M. Lein, W. Henke, D. Schnorr, B. Brux, S. Loening, Freies prostataspezifisches Antigen im Serum: eine Meßgröße zur Diskrimination zwischen Patienten mit Prostatakarzinom und benigner Prostatahyperplasie, J. Lab. Med. 20 (1996) 7–12.
[50] C. Stephan, K. Jung, M. Lein, P. Sinha, D. Schnorr, S.A. Loening, Molecular forms of prostate-specific antigen and human kallikrein 2 as promising tools for early diagnosis of prostate cancer, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 1133–1147.
[51] W.J. Catalona, A.W. Partin, J.A. Finlay, D.W. Chan, H.G. Rittenhouse, R.L. Wolfert, D.L. Woodrum, Use of percentage of free prostate-specific antigen to identify men at high risk of prostate cancer when PSA levels are 2.51 to 4 ng/mL and digital rectal examination is not suspicious for prostate cancer: an alternative model, Urology 54 (1999) 220–224.
[52] K. Jung, C. Stephan, U. Elgeti, M. Lein, B. Brux, G. Kristiansen, B. Rudolph, S. Hauptmann, D. Schnorr, S.A. Loening, Molecular forms of prostate-specific antigen in serum with concentrations of total prostate-specific antigen <4 μg/l — are they useful tools for early detection and screening of prostate cancer? Int. J. Cancer 93 (2001) 759–765.
[53] K.A. Roehl, J.A. Antenor, W.J. Catalona, Robustness of free prostate specific antigen measurements to reduce unnecessary biopsies in the 2.6 to 4.0 ng./ml. range, J. Urol. 168 (2002) 922–925.
[54] R. Lee, A.R. Localio, K. Armstrong, S.B. Malkowicz, J.S. Schwartz, A meta-analysis of the performance characteristics of the free prostate-specific antigen test, Urology 67 (2006) 762–768.
[55] C. Stephan, M. Lein, K. Jung, D. Schnorr, S.A. Loening, The influence of prostate volume on the ratio of free to total prostate specific antigen in serum of patients with prostate carcinoma and benign prostate hyperplasia, Cancer 79 (1997) 104–109.
[56] C. Stephan, M. Lein, K. Jung, D. Schnorr, S.A. Loening, Can prostate specific antigen derivatives reduce the frequency of unnecessary prostate biopsies?[Letter], J. Urol. 157 (1997) 1371.

[57] C.T. Lee, P.T. Scardino, Percent free prostate-specific antigen for first-time prostate biopsy, Urology 57 (2001) 594–598.
[58] P. Finne, A. Auvinen, J. Aro, H. Juusela, L. Maattanen, S. Rannikko, M. Hakama, T.L. Tammela, U.H. Stenman, Estimation of prostate cancer risk on the basis of total and free prostate-specific antigen, prostate volume and digital rectal examination, Eur. Urol. 41 (2002) 619–626.
[59] P. Finne, A. Auvinen, L. Maattanen, T.L. Tammela, M. Ruutu, H. Juusela, P. Martikainen, M. Hakama, U.H. Stenman, Diagnostic value of free prostate-specific antigen among men with a prostate-specific antigen level of <3.0 microg per liter, Eur. Urol. 54 (2008) 362–370.
[60] S.B. Larsen, K. Brasso, P. Iversen, J. Christensen, M. Christiansen, S. Carlsson, H. Lilja, S. Friis, A. Tjonneland, S.O. Dalton, Baseline prostate-specific antigen measurements and subsequent prostate cancer risk in the Danish Diet, Cancer and Health cohort, Eur. J. Cancer 49 (2013) 3041–3048.
[61] H. Lilja, D. Ulmert, T. Bjork, C. Becker, A.M. Serio, J.A. Nilsson, P.A. Abrahamsson, A.J. Vickers, G. Berglund, Long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using prostate kallikreins measured at age 44 to 50 years, J. Clin. Oncol. 25 (2007) 431–436.
[62] A. Benchikh, C. Savage, A. Cronin, G. Salama, A. Villers, H. Lilja, A. Vickers, A panel of kallikrein markers can predict outcome of prostate biopsy following clinical work-up: an independent validation study from the European Randomized Study of Prostate Cancer screening, France, BMC Cancer 10 (2010) 635.
[63] A. Vickers, A. Cronin, M. Roobol, C. Savage, M. Peltola, K. Pettersson, P.T. Scardino, F. Schroder, H. Lilja, Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication, J. Clin. Oncol. 28 (2010) 2493–2498.
[64] C. Stephan, H. Cammann, H.A. Meyer, M. Lein, K. Jung, PSA and new biomarkers within multivariate models to improve early detection of prostate cancer, Cancer Lett. 249 (2007) 18–29.
[65] R.J. Babaian, H. Fritsche, A. Ayala, V. Bhadkamkar, D.A. Johnston, W. Naccarato, Z. Zhang, 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 (2000) 1000–1006.
[66] G.D. Carlson, C.B. Calvanese, A.W. Partin, An algorithm combining age, total prostate-specific antigen (PSA), and percent free PSA to predict prostate cancer: results on 4298 cases, Urology 52 (1998) 455–461.
[67] P. Finne, R. Finne, A. Auvinen, H. Juusela, J. Aro, L. Maattanen, M. Hakama, S. Rannikko, T.L. Tammela, U. Stenman, Predicting the outcome of prostate biopsy in screen-positive men by a multilayer perceptron network, Urology 56 (2000) 418–422.
[68] P. Finne, R. Finne, C. Bangma, J. Hugosson, M. Hakama, A. Auvinen, U.H. Stenman, 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 (2004) 310–315.
[69] W. Horninger, G. Bartsch, P.B. Snow, J.M. Brandt, A.W. Partin, The problem of cutoff levels in a screened population, Cancer 91 (2001) 1667–1672.
[70] M. Remzi, T. Anagnostou, V. Ravery, A. Zlotta, C. Stephan, M. Marberger, B. Djavan, An artificial neural network to predict the outcome of repeat prostate biopsies, Urology 62 (2003) 456–460.
[71] C. Stephan, H. Cammann, A. Semjonow, E.P. Diamandis, L.F.A. Wymenga, M. Lein, P. Sinha, S.A. Loening, K. Jung, Multicenter evaluation of an artificial neural network to increase prostate cancer detection rate and reduce unnecessary biopsies, Clin. Chem. 48 (2002) 1279–1287.
[72] A. Virtanen, M. Gomari, R. Kranse, U.H. Stenman, Estimation of prostate cancer probability by logistic regression: free and total prostate-specific antigen, digital rectal examination, and heredity are significant variables, Clin. Chem. 45 (1999) 987–994.
[73] C. Stephan, H. Cammann, H.A. Meyer, C. Muller, S. Deger, M. Lein, K. Jung, An artificial neural network for five different assay systems of prostate-specific antigen in prostate cancer diagnostics, BJU Int. 102 (2008) 799–805.
[74] P.I. Karakiewicz, S. Benayoun, M.W. Kattan, P. Perrotte, L. Valiquette, P.T. Scardino, I. Cagiannos, H. Heinzer, S. Tanguay, A.G. Aprikian, H. Huland, M. Graefen, Development and validation of a nomogram predicting the outcome of prostate biopsy based on patient age, digital rectal examination and serum prostate specific antigen, J. Urol. 173 (2005) 1930–1934.
[75] S.D. Mikolajczyk, L.S. Millar, T.J. Wang, H.G. Rittenhouse, L.S. Marks, W. Song, T.M. Wheeler, K.M. Slawin, A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue, Cancer Res. 60 (2000) 756–759.
[76] S.D. Mikolajczyk, K.M. Marker, L.S. Millar, A. Kumar, M.S. Saedi, J.K. Payne, C.L. Evans, C.L. Gasior, H.J. Linton, P. Carpenter, H.G. Rittenhouse, A truncated precursor form of prostate-specific antigen is a more specific serum marker of prostate cancer, Cancer Res. 61 (2001) 6958–6963.
[77] P. Nurmikko, V. Vaisanen, T. Piironen, S. Lindgren, H. Lilja, K. Pettersson, 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 (2000) 1610–1618.
[78] P. Nurmikko, K. Pettersson, T. Piironen, J. Hugosson, H. Lilja, 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 (2001) 1415–1423.
[79] J. Peter, C. Unverzagt, T.N. Krogh, O. Vorm, W. Hoesel, Identification of precursor forms of free prostate-specific antigen in serum of prostate cancer patients by immunosorption and mass spectrometry, Cancer Res. 61 (2001) 957–962.
[80] S.D. Mikolajczyk, L.S. Marks, A.W. Partin, H.G. Rittenhouse, Free prostate-specific antigen in serum is becoming more complex, Urology 59 (2002) 797–802.
[81] H.J. Linton, L.S. Marks, L.S. Millar, C.L. Knott, H.G. Rittenhouse, S.D. Mikolajczyk, Benign prostate-specific antigen (BPSA) in serum is increased in benign prostate disease, Clin. Chem. 49 (2003) 253–259.
[82] C. Stephan, H. Cammann, S. Deger, M. Schrader, H.A. Meyer, K. Miller, M. Lein, K. Jung, Benign prostatic hyperplasia-associated free prostate-specific antigen improves detection of prostate cancer in an artificial neural network, Urology 74 (2009) 873–877.
[83] T. Steuber, P. Nurmikko, A. Haese, K. Pettersson, M. Graefen, P. Hammerer, H. Huland, H. Lilja, Discrimination of benign from malignant prostatic disease by selective measurements of single chain, intact free prostate specific antigen, J. Urol. 168 (2002) 1917–1922.
[84] S.D. Mikolajczyk, W.J. Catalona, C.L. Evans, H.J. Linton, L.S. Millar, K.M. Marker, D. Katir, A. Amirkhan, H.G. Rittenhouse, Proenzyme forms of prostate-specific antigen in serum improve the detection of prostate cancer, Clin. Chem. 50 (2004) 1017–1025.
[85] W.J. Catalona, G. Bartsch, H.G. Rittenhouse, C.L. Evans, H.J. Linton, A. Amirkhan, W. Horninger, H. Klocker, S.D. Mikolajczyk, Serum pro prostate 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 (2003) 2181–2185.
[86] W.J. Catalona, A.W. Partin, M.G. Sanda, J.T. Wei, G.G. Klee, C.H. Bangma, K.M. Slawin, L.S. Marks, S. Loeb, D.L. Broyles, S.S. Shin, A.B. Cruz, D.W. Chan, L.J. Sokoll, W.L. Roberts, R.H. van Schaik, I.A. Mizrahi, A multicenter study of [−2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range, J. Urol. 185 (2011) 1650–1655.
[87] L.J. Sokoll, Y. Wang, Z. Feng, J. Kagan, A.W. Partin, M.G. Sanda, I.M. Thompson, D.W. Chan, [−2]proenzyme prostate specific antigen for prostate cancer detection: a national cancer institute early detection research network validation study, J. Urol. 180 (2008) 539–543.
[88] C. Stephan, A.M. Kahrs, H. Cammann, M. Lein, M. Schrader, S. Deger, K. Miller, K. Jung, A [−2]proPSA-based artificial neural network significantly improves differentiation between prostate cancer and benign prostatic diseases, Prostate 69 (2009) 198–207.
[89] C. Stephan, S. Vincendeau, A. Houlgatte, H. Cammann, K. Jung, A. Semjonow, Multicenter evaluation of [−2]proprostate-specific antigen and the prostate health index for detecting prostate cancer, Clin. Chem. 59 (2013) 306–314.
[90] F.H. Jansen, R.H. van Schaik, J. Kurstjens, W. Horninger, H. Klocker, J. Bektic, M.F. Wildhagen, M.J. Roobol, C.H. Bangma, G. Bartsch, Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves diagnostic accuracy in prostate cancer detection, Eur. Urol. 57 (2010) 921–927.
[91] B.V. Le, C.R. Griffin, S. Loeb, G.F. Carvalhal, D. Kan, N.A. Baumann, W.J. Catalona, [−2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study, J. Urol. 183 (2010) 1355–1359.
[92] M. Lazzeri, A. Haese, A. de la Taille, R.J. Palou, T. McNicholas, G. Lughezzani, V. Scattoni, V. Bini, M. Freschi, A. Sussman, B. Ghaleb, C.P. Le, B.J. Alberola, F.S. Esquena, M. Graefen, G. Guazzoni, Serum isoform [−2]proPSA derivatives significantly improve prediction of prostate cancer at initial biopsy in a total PSA range of 2–10 ng/ml: a multicentric European study, Eur. Urol. 63 (2013) 986–994.
[93] L.J. Sokoll, M.G. Sanda, Z. Feng, J. Kagan, I.A. Mizrahi, D.L. Broyles, A.W. Partin, S. Srivastava, I.M. Thompson, J.T. Wei, Z. Zhang, D.W. Chan, A prospective, multicenter, National Cancer Institute Early Detection Research Network study of [−2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness, Cancer Epidemiol. Biomarkers Prev. 19 (2010) 1193–1200.
[94] S. Hori, J.S. Blanchet, J. McLoughlin, 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 Int. 112 (2013) 717–728.
[95] E. Heijnsdijk, J. Huang, D. Denham, H.J. De Koning, The cost-effectiveness of prostate cancer detection using the prostate health index phi, Eur. Urol. Suppl. 11 (2012) e260.
[96] X. Filella, N. Gimenez, Evaluation of [−2] proPSA and Prostate Health Index (phi) for the detection of prostate cancer: a systematic review and meta-analysis, Clin. Chem. Lab. Med. 51 (2013) 729–739.
[97] G. Guazzoni, M. Lazzeri, L. Nava, G. Lughezzani, A. Larcher, V. Scattoni, G.M. Gadda, V. Bini, A. Cestari, N.M. Buffi, M. Freschi, P. Rigatti, F. Montorsi, Preoperative prostate-specific antigen isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for prostate cancer, Eur. Urol. 61 (2012) 455–466.
[98] M. Ferro, D. Bruzzese, S. Perdona, C. Mazzarella, A. Marino, A. Sorrentino, A. Di Carlo, R. Autorino, G. Di Lorenzo, C. Buonerba, V. Altieri, A. Mariano, V. Macchia, D. Terracciano, Predicting prostate biopsy outcome: prostate health index (phi) and prostate cancer antigen 3 (PCA3) are useful biomarkers, Clin. Chim. Acta 413 (2012) 1274–1278.
[99] M.J. Roobol, S.V. Carlsson, Risk stratification in prostate cancer screening, Nat. Rev. Urol. 10 (2013) 38–48.
[100] S. Loeb, Prostate Health Index (PHI): golden bullet or just another prostate cancer marker? Eur. Urol. 63 (2013) 995–996.
[101] H.G. Rittenhouse, J.A. Finlay, S.D. Mikolajczyk, A.W. Partin, Human Kallikrein 2 (hK2) and prostate-specific antigen (PSA): two closely related, but distinct, kallikreins in the prostate, Crit. Rev. Clin. Lab. Sci. 35 (1998) 275–368.
[102] G.M. Yousef, E.P. Diamandis, The new human tissue kallikrein gene family: structure, function, and association to disease, Endocr. Rev. 22 (2001) 184–204.
[103] A. Lundwall, V. Band, M. Blaber, J.A. Clements, Y. Courty, E.P. Diamandis, H. Fritz, H. Lilja, J. Malm, L.J. Maltais, A.Y. Olsson, C. Petraki, A. Scorilas, G. Sotiropoulou, U.H.

Stenman, C. Stephan, M. Talieri, G.M. Yousef, A comprehensive nomenclature for serine proteases with homology to tissue kallikreins, Biol. Chem. 387 (2006) 637–641.
[104] A. Kumar, S.D. Mikolajczyk, A.S. Goel, L.S. Millar, M.S. Saedi, Expression of pro form of prostate-specific antigen by mammalian cells and its conversion to mature, active form by human kallikrein 2, Cancer Res. 57 (1997) 3111–3114.
[105] J. Lövgren, K. Rajakoski, M. Karp, A. Lundwall, H. Lilja, Activation of the zymogen form of prostate-specific antigen by human glandular kallikrein 2, Biochem. Biophys. Res. Commun. 238 (1997) 549–555.
[106] T.K. Takayama, K. Fujikawa, E.W. Davie, Characterization of the precursor of prostate-specific antigen. Activation by trypsin and by human glandular kallikrein, J. Biol. Chem. 272 (1997) 21582–21588.
[107] C. Becker, T. Piironen, K. Pettersson, T. Björk, K.J. Wojno, J.E. Oesterling, H. Lilja, Discrimination of men with prostate cancer from those with benign disease by measurements of human glandular kallikrein 2 (HK2) in serum, J. Urol. 163 (2000) 311–316.
[108] M.K. Kwiatkowski, F. Recker, T. Piironen, K. Pettersson, T. Otto, M. Wernli, R. Tscholl, 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 (1998) 360–365.
[109] A. Magklara, A. Scorilas, W.J. Catalona, E.P. Diamandis, The combination of human glandular kallikrein and free prostate-specific antigen (PSA) enhances discrimination between prostate cancer and benign prostatic hyperplasia in patients with moderately increased total PSA, Clin. Chem. 45 (1999) 1960–1966.
[110] R.K. Nam, E.P. Diamandis, A. Toi, J. Trachtenberg, A. Magklara, A. Scorilas, P.A. Papnastasiou, M.A. Jewett, S.A. Narod, Serum human glandular kallikrein-2 protease levels predict the presence of prostate cancer among men with elevated prostate-specific antigen, J. Clin. Oncol. 18 (2000) 1036–1042.
[111] A.W. Partin, W.J. Catalona, J.A. Finlay, C. Darte, D.J. Tindall, C.Y. Young, G.G. Klee, D. W. Chan, H.G. Rittenhouse, R.L. Wolfert, D.L. Woodrum, Use of human glandular kallikrein 2 for the detection of prostate cancer: preliminary analysis, Urology 54 (1999) 839–845.
[112] A. Haese, J. Noldus, M. Graefen, T. Steuber, H. Huland, C. Becker, H. Lilja, Human glandular kallikrein 2 (hK2) is superior to total PSA in predicting organ-confined disease and pathological stage of clinically localized prostate cancer with a total PSA <10 ng/ml, J. Urol. 165 (2001) 234 (Suppl.).
[113] A. Haese, M. Graefen, T. Steuber, C. Becker, K. Pettersson, T. Piironen, J. Noldus, H. Huland, H. Lilja, Human glandular kallikrein 2 levels in serum for discrimination of pathologically organ-confined from locally-advanced prostate cancer in total PSA-levels below 10 ng/ml, Prostate 49 (2001) 101–109.
[114] F. Recker, M.K. Kwiatkowski, T. Piironen, K. Pettersson, A. Huber, G. Lümmen, R. Tscholl, Human glandular kallikrein as a tool to improve discrimination of poorly differentiated and non-organ-confined prostate cancer compared with prostate-specific antigen, Urology 55 (2000) 481–485.
[115] C. Stephan, K. Jung, T. Nakamura, G.M. Yousef, G. Kristiansen, E.P. Diamandis, Serum human glandular kallikrein 2 (hK2) for distinguishing stage and grade of prostate cancer, Int. J. Urol. 13 (2006) 238–243.
[116] C.H. Bangma, M.F. Wildhagen, G. Yurdakul, F.H. Schroder, B.G. Blijenberg, The value of (−7, −5)pro-prostate-specific antigen and human kallikrein-2 as serum markers for grading prostate cancer, BJU Int. 93 (2004) 720–724.
[117] A. Haese, M. Graefen, C. Becker, J. Noldus, J. Katz, I. Cagiannos, M. Kattan, P.T. Scardino, E. Huland, H. Huland, H. Lilja, The role of human glandular kallikrein 2 for prediction of pathologically organ confined prostate cancer, Prostate 54 (2003) 181–186.
[118] C. Stephan, K. Jung, A. Soosaipillai, G.M. Yousef, H. Cammann, H. Meyer, C. Xu, E.P. Diamandis, Clinical utility of human glandular kallikrein 2 within a neural network for prostate cancer detection, BJU Int. 96 (2005) 521–527.
[119] T.K. Takayama, B.A. McMullen, P.S. Nelson, M. Matsumura, K. Fujikawa, Characterization of hK4 (prostase), a prostate-specific serine protease: activation of the precursor of prostate specific antigen (pro-PSA) and single-chain urokinase-type plasminogen activator and degradation of prostatic acid phosphatase, Biochemistry 40 (2001) 15341–15348.
[120] T. Nakamura, A. Scorilas, C. Stephan, K. Jung, A.R. Soosaipillai, E.P. Diamandis, The usefulness of serum human kallikrein 11 for discriminating between prostate cancer and benign prostatic hyperplasia, Cancer Res. 63 (2003) 6543–6546.
[121] C. Stephan, H.A. Meyer, H. Cammann, T. Nakamura, E.P. Diamandis, K. Jung, Improved prostate cancer detection with a human kallikrein 11 and percentage free PSA-based artificial neural network, Biol. Chem. 387 (2006) 801–805.
[122] C. Planque, L. Li, Y. Zheng, A. Soosaipillai, K. Reckamp, D. Chia, E.P. Diamandis, L. Goodglick, A multiparametric serum kallikrein panel for diagnosis of non-small cell lung carcinoma, Clin. Cancer Res. 14 (2008) 1355–1362.
[123] K.L. Ewan, X. Li, B.K. Cheikh Saad, M. Pedneault, C.W. Chu, Human kallikrein 10 ELISA development and validation in breast cancer sera, Clin. Biochem. 40 (2007) 1057–1062.
[124] C. Stephan, G.M. Yousef, A. Scorilas, K. Jung, M. Jung, G. Kristiansen, S. Hauptmann, B.S. Bharaj, T. Nakamura, S.A. Loening, E.P. Diamandis, Quantitative analysis of kallikrein 15 (KLK15) gene expression in prostate tissue, J. Urol. 169 (2003) 361–364.
[125] K. Mavridis, K. Stravodimos, A. Scorilas, Quantified KLK15 gene expression levels discriminate prostate cancer from benign tumors and constitute a novel independent predictor of disease progression, Prostate 73 (2013) 1191–1201.
[126] A. Rabien, F.R. Fritzsche, M. Jung, A. Tolle, E.P. Diamandis, K. Miller, K. Jung, G. Kristiansen, C. Stephan, KLK15 is a prognostic marker for progression-free survival in patients with radical prostatectomy, Int. J. Cancer 127 (2010) 2386–2394.
[127] J.L. Shaw, L. Grass, G. Sotiropoulou, E.P. Diamandis, Development of an immunofluorometric assay for human kallikrein 15 (KLK15) and identification of KLK15 in tissues and biological fluids, Clin. Biochem. 40 (2007) 104–110.
[128] M. Paliouras, E.P. Diamandis, The kallikrein world: an update on the human tissue kallikreins, Biol. Chem. 387 (2006) 643–652.
[129] D.L. Thorek, M.J. Evans, S.V. Carlsson, D. Ulmert, H. Lilja, Prostate-specific kallikrein-related peptidases and their relation to prostate cancer biology and detection. Established relevance and emerging roles, Thromb. Haemost. 110 (2013) 484–492.
[130] C. Stephan, H. Rittenhouse, H. Cammann, M. Lein, M. Schrader, S. Deger, K. Miller, K. Jung, New markers and multivariate models for prostate cancer detection, Anticancer Res. 29 (2009) 2589–2600.
[131] E.P. Diamandis, EPCA-2: a highly specific serum marker for prostate cancer, Clin. Biochem. 45 (2012) 600.
[132] D.A. Brown, C. Stephan, R.L. Ward, M. Law, M. Hunter, A.R. Bauskin, J. Amin, K. Jung, E.P. Diamandis, G.M. Hampton, P.J. Russell, G.G. Giles, S.N. Breit, Measurement of serum levels of macrophage inhibitory cytokine 1 combined with prostate-specific antigen improves prostate cancer diagnosis, Clin. Cancer Res. 12 (2006) 89–96.
[133] D.A. Brown, F. Lindmark, P. Stattin, K. Balter, H.O. Adami, S.L. Zheng, J. Xu, W.B. Isaacs, H. Gronberg, S.N. Breit, F.E. Wiklund, Macrophage inhibitory cytokine 1: a new prognostic marker in prostate cancer, Clin. Cancer Res. 15 (2009) 6658–6664.
[134] K.L. Meyer-Siegler, M.A. Bellino, M. Tannenbaum, Macrophage migration inhibitory factor evaluation compared with prostate specific antigen as a biomarker in patients with prostate carcinoma, Cancer 94 (2002) 1449–1456.
[135] A. Michael, C. Stephan, G. Kristiansen, M. Burckhardt, S.A. Loening, D. Schnorr, K. Jung, Diagnostic validity of macrophage migration inhibitory factor in serum of patients with prostate cancer: a re-evaluation, Prostate 62 (2005) 34–39.
[136] C. Stephan, C. Xu, D.A. Brown, S.N. Breit, A. Michael, T. Nakamura, E.P. Diamandis, H. Meyer, H. Cammann, K. Jung, Three new serum markers for prostate cancer detection within a percent free PSA-based artificial neural network, Prostate 66 (2006) 651–659.
[137] A. Hermani, J. Hess, B. De Servi, S. Medunjanin, R. Grobholz, L. Trojan, P. Angel, D. Mayer, Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer, Clin. Cancer Res. 11 (2005) 5146–5152.
[138] S. Ludwig, C. Stephan, M. Lein, S.A. Loening, K. Jung, S100A8, S100A9, and the S100A8/A9 complex in circulating blood are not associated with prostate cancer risk—A re-evaluation study, Prostate 67 (2007) 1301–1307.
[139] G. Lucarelli, M. Rutigliano, C. Bettocchi, S. Palazzo, A. Vavallo, V. Galleggiante, S. Trabucco, D. Di Clemente, F.P. Selvaggi, M. Battaglia, P. Ditonno, Spondin-2, a secreted extracellular matrix protein, is a novel diagnostic biomarker for prostate cancer, J. Urol. 190 (2013) 2271–2277.
[140] V. Balan, Y. Wang, P. Nangia-Makker, D. Kho, M. Bajaj, D. Smith, L. Heilbrun, A. Raz, E. Heath, Galectin-3: a possible complementary marker to the PSA blood test, Oncotarget 4 (2013) 542–549.
[141] X. Qian, C. Li, B. Pang, M. Xue, J. Wang, J. Zhou, Spondin-2 (SPON2), a more prostate-cancer-specific diagnostic biomarker, PLoS One 7 (2012) e37225.
[142] C.E. Barbieri, Evolution of novel biomarkers for detection of prostate cancer, J. Urol. 190 (2013) 1970–1971.
[143] S. Koutros, T.E. Meyer, S.D. Fox, H.J. Issaq, T.D. Veenstra, W.Y. Huang, K. Yu, D. Albanes, L.W. Chu, G. Andriole, R.N. Hoover, A.W. Hsing, S.I. Berndt, Prospective evaluation of serum sarcosine and risk of prostate cancer in the prostate, lung, colorectal and ovarian cancer screening trial, Carcinogenesis 34 (2013) 2281–2285.
[144] S. de Vogel, A. Ulvik, K. Meyer, P.M. Ueland, O. Nygard, S.E. Vollset, G.S. Tell, J.F. Gregory III, S. Tretli, T. Bjorge, Sarcosine and other metabolites along the choline oxidation pathway in relation to prostate cancer–a large nested case–control study within the JANUS cohort in Norway, Int. J. Cancer 134 (2014) 197–206.
[145] E.A. Struys, A.C. Heijboer, J. van Moorselaar, C. Jakobs, M.A. Blankenstein, Serum sarcosine is not a marker for prostate cancer, Ann. Clin. Biochem. 47 (2010) 282.
[146] G. Lucarelli, P. Ditonno, C. Bettocchi, M. Spilotros, M. Rutigliano, A. Vavallo, V. Galleggiante, M. Fanelli, A.M. Larocca, C.A. Germinario, E. Maiorano, F.P. Selvaggi, M. Battaglia, Serum sarcosine is a risk factor for progression and survival in patients with metastatic castration-resistant prostate cancer, Future Oncol. 9 (2013) 899–907.
[147] G. Lucarelli, M. Fanelli, A.M. Larocca, C.A. Germinario, M. Rutigliano, A. Vavallo, F.P. Selvaggi, C. Bettocchi, M. Battaglia, P. Ditonno, Serum sarcosine increases the accuracy of prostate cancer detection in patients with total serum PSA less than 4.0 ng/ml, Prostate 72 (2012) 1611–1621.
[148] L. Bohm, A.M. Serafin, P. Fernandez, G. Van der Watt, P.J. Bouic, J. Harvey, Plasma sarcosine does not distinguish early and advanced stages of prostate cancer, S. Afr. Med. J. 102 (2012) 677–679.
[149] A. Sreekumar, L.M. Poisson, T.M. Rajendiran, A.P. Khan, Q. Cao, J. Yu, B. Laxman, R. Mehra, R.J. Lonigro, Y. Li, M.K. Nyati, A. Ahsan, S. Kalyana-Sundaram, B. Han, X. Cao, J. Byun, G.S. Omenn, D. Ghosh, S. Pennathur, D.C. Alexander, A. Berger, J.R. Shuster, J.T. Wei, S. Varambally, C. Beecher, A.M. Chinnaiyan, Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression, Nature 457 (2009) 910–914.
[150] F. Jentzmik, C. Stephan, K. Miller, M. Schrader, A. Erbersdobler, G. Kristiansen, M. Lein, K. Jung, Sarcosine in urine after digital rectal examination fails as a marker in prostate cancer detection and identification of aggressive tumours, Eur. Urol. 58 (2010) 12–18.
[151] F. Jentzmik, C. Stephan, M. Lein, K. Miller, B. Kamlage, B. Bethan, G. Kristiansen, K. Jung, Sarcosine in prostate cancer tissue is not a differential metabolite for prostate cancer aggressiveness and biochemical progression, J. Urol. 185 (2011) 706–711.
[152] J.B. de Kok, G.W. Verhaegh, R.W. Roelofs, D. Hessels, L.A. Kiemeney, T.W. Aalders, D. W. Swinkels, J.A. Schalken, DD3(PCA3), a very sensitive and specific marker to detect prostate tumors, Cancer Res. 62 (2002) 2695–2698.

[153] L.J. Sokoll, W. Ellis, P. Lange, J. Noteboom, D.J. Elliott, I.L. Deras, A. Blase, S. Koo, M. Sarno, H. Rittenhouse, J. Groskopf, R.L. Vessella, A multicenter evaluation of the PCA3 molecular urine test: pre-analytical effects, analytical performance, and diagnostic accuracy, Clin. Chim. Acta 389 (2008) 1–6.
[154] N. Cernei, Z. Heger, J. Gumulec, O. Zitka, M. Masarik, P. Babula, T. Eckschlager, M. Stiborova, R. Kizek, V. Adam, Sarcosine as a potential prostate cancer biomarker—a review, Int. J. Mol. Sci. 14 (2013) 13893–13908.
[155] D. Hessels, J.M. Klein Gunnewiek, I. van Oort, H.F. Karthaus, G.J. van Leenders, B. van Balken, L.A. Kiemeney, J.A. Witjes, J.A. Schalken, DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer, Eur. Urol. 44 (2003) 8–15.
[156] X. Filella, L. Foj, M. Mila, J.M. Auge, R. Molina, W. Jimenez, PCA3 in the detection and management of early prostate cancer, Tumour Biol. 34 (2013) 1337–1347.
[157] J. Groskopf, S.M. Aubin, I.L. Deras, A. Blase, S. Bodrug, C. Clark, S. Brentano, J. Mathis, J. Pham, T. Meyer, M. Cass, P. Hodge, M.L. Macairan, L.S. Marks, H. Rittenhouse, APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer, Clin. Chem. 52 (2006) 1089–1095.
[158] A. Haese, A. de la Taille, H. Van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, C.C. Abbou, M. Remzi, M. Tinzl, S. Feyerabend, A.B. Stillebroer, M.P. van Gils, J.A. Schalken, Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy, Eur. Urol. 54 (2008) 1081–1088.
[159] I.L. Deras, S.M. Aubin, A. Blase, J.R. Day, S. Koo, A.W. Partin, W.J. Ellis, L.S. Marks, Y. Fradet, H. Rittenhouse, J. Groskopf, PCA3: a molecular urine assay for predicting prostate biopsy outcome, J. Urol. 179 (2008) 1587–1592.
[160] M.J. Roobol, F.H. Schroder, G.L. van Leenders, D. Hessels, R.C. van den Bergh, T. Wolters, P.J. van Leeuwen, Performance of prostate cancer antigen 3 (PCA3) and prostate-specific antigen in Prescreened men: reproducibility and detection characteristics for prostate cancer patients with high PCA3 scores (>/=100, Eur. Urol. 58 (2010) 893–899.
[161] L.A. Bradley, G.E. Palomaki, S. Gutman, D. Samson, N. Aronson, Comparative effectiveness review: prostate cancer antigen 3 testing for the diagnosis and management of prostate cancer, J. Urol. 190 (2013) 389–398.
[162] M.J. Roobol, Contemporary role of prostate cancer gene 3 in the management of prostate cancer, Curr. Opin. Urol. 21 (2011) 225–229.
[163] M. Truong, B. Yang, D.F. Jarrard, Toward the detection of prostate cancer in urine: a critical analysis, J. Urol. 189 (2013) 422–429.
[164] M. Auprich, A. Bjartell, F.K. Chun, A. de la Taille, S.J. Freedland, A. Haese, J. Schalken, A. Stenzl, B. Tombal, H. van der Poel, Contemporary role of prostate cancer antigen 3 in the management of prostate cancer, Eur. Urol. 60 (2011) 1045–1054.
[165] M. Ferro, D. Bruzzese, S. Perdona, A. Marino, C. Mazzarella, G. Perruolo, V. D'Esposito, V. Cosimato, C. Buonerba, G. Di Lorenzo, G. Musi, O. De Cobelli, F.K. Chun, D. Terracciano, Prostate Health Index (Phi) and Prostate Cancer Antigen 3 (PCA3) significantly improve prostate cancer detection at initial biopsy in a total PSA range of 2–10 ng/ml, PLoS One 8 (2013) e67687.
[166] M. Auprich, A. Haese, J. Walz, K. Pummer, A. de la Taille, M. Graefen, T. de Reijke, M. Fisch, P. Kil, P. Gontero, J. Irani, F.K. Chun, External validation of urinary PCA3-based nomograms to individually predict prostate biopsy outcome, Eur. Urol. 58 (2010) 727–732.
[167] D. Hessels, M.P. van Gils, O. van Hooij, S.A. Jannink, J.A. Witjes, G.W. Verhaegh, J.A. Schalken, Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer, Prostate 70 (2010) 10–16.
[168] L.S. Marks, Y. Fradet, I.L. Deras, A. Blase, J. Mathis, S.M. Aubin, A.T. Cancio, M. Desaulniers, W.J. Ellis, H. Rittenhouse, J. Groskopf, PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy, Urology 69 (2007) 532–535.
[169] D.P. Ankerst, J. Groskopf, J.R. Day, A. Blase, H. Rittenhouse, B.H. Pollock, C. Tangen, D. Parekh, R.J. Leach, I. Thompson, Predicting prostate cancer risk through incorporation of prostate cancer gene 3, J. Urol. 180 (2008) 1303–1308.
[170] S.M. Aubin, J. Reid, M.J. Sarno, A. Blase, J. Aussie, H. Rittenhouse, R.S. Rittmaster, G.L. Andriole, J. Groskopf, Prostate cancer gene 3 score predicts prostate biopsy outcome in men receiving dutasteride for prevention of prostate cancer: results from the REDUCE trial, Urology 78 (2011) 380–385.
[171] E. Bollito, S. De Luca, M. Cicilano, R. Passera, S. Grande, C. Maccagnano, S. Cappia, A. Milillo, F. Montorsi, R.M. Scarpa, M. Papotti, D.F. Randone, Prostate cancer gene 3 urine assay cutoff in diagnosis of prostate cancer: a validation study on an Italian patient population undergoing first and repeat biopsy, Anal. Quant. Cytol. Histol. 34 (2012) 96–104.
[172] F.K. Chun, A. de la Taille, H. Van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, C.C. Abbou, A.B. Stillebroer, M.P. van Gils, J.A. Schalken, Y. Fradet, L.S. Marks, W. Ellis, A.W. Partin, A. Haese, Prostate cancer gene 3 (PCA3): development and internal validation of a novel biopsy nomogram, Eur. Urol. 56 (2009) 659–668.
[173] M.C. Gittelman, B. Hertzman, J. Bailen, T. Williams, I. Koziol, R.J. Henderson, M. Efros, M. Bidair, J.F. Ward, PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study, J. Urol. 190 (2013) 64–69.
[174] J. Hansen, M. Auprich, S.A. Ahyai, A. de la Taille, H. Van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, M. Fisch, C.C. Abbou, J.A. Schalken, Y. Fradet, L.S. Marks, W. Ellis, A.W. Partin, K. Pummer, M. Graefen, A. Haese, J. Walz, A. Briganti, S.F. Shariat, F.K. Chun, Initial prostate biopsy: development and internal validation of a biopsy-specific nomogram based on the prostate cancer antigen 3 assay, Eur. Urol. 63 (2013) 201–209.
[175] A. Ochiai, K. Okihara, K. Kamoi, T. Oikawa, T. Shimazui, S. Murayama, K. Tomita, T. Umekawa, H. Uemura, T. Miki, Clinical utility of the prostate cancer gene 3 (PCA3) urine assay in Japanese men undergoing prostate biopsy, BJU Int. 111 (2013) 928–933.
[176] S. Perdona, V. Cavadas, G. Di Lorenzo, R. Damiano, G. Chiappetta, P. Del Prete, R. Franco, G. Azzarito, S. Scala, C. Arra, M. De Sio, R. Autorino, Prostate cancer detection in the "grey area" of prostate-specific antigen below 10 ng/ml: head-to-head comparison of the updated PCPT calculator and Chun's nomogram, two risk estimators incorporating prostate cancer antigen 3, Eur. Urol. 59 (2011) 81–87.
[177] M.J. Roobol, F.H. Schroder, P. van Leeuwen, T. Wolters, R.C. van den Bergh, G.J. van Leenders, D. Hessels, Performance of the prostate cancer antigen 3 (PCA3) gene and prostate-specific antigen in prescreened men: exploring the value of PCA3 for a first-line diagnostic test, Eur. Urol. 58 (2010) 475–481.
[178] C. Stephan, K. Jung, A. Semjonow, K. Schulze-Forster, H. Cammann, X. Hu, H.A. Meyer, M. Bogemann, K. Miller, F. Friedersdorff, Comparative assessment of urinary prostate cancer antigen 3 and TMPRSS2:ERG gene fusion with the serum [−2]proprostate-specific antigen-based prostate health index for detection of prostate cancer, Clin. Chem. 59 (2013) 280–288.
[179] B. Tombal, G.L. Andriole, A. de la Taille, P. Gontero, A. Haese, M. Remzi, M. Speakman, L. Smets, H. Stoevelaar, Clinical judgment versus biomarker prostate cancer gene 3: which is best when determining the need for repeat prostate biopsy? Urology 81 (2013) 998–1004.
[180] R.R. Goode, S.J. Marshall, M. Duff, E. Chevli, K.K. Chevli, Use of PCA3 in detecting prostate cancer in initial and repeat prostate biopsy patients, Prostate 73 (2013) 48–53.
[181] G. Ploussard, A. Haese, H. Van Poppel, M. Marberger, A. Stenzl, P.F. Mulders, H. Huland, L. Bastien, C.C. Abbou, M. Remzi, M. Tinzl, S. Feyerabend, A.B. Stillebroer, M.P. van Gils, J.A. Schalken, A. de la Taille, The prostate cancer gene 3 (PCA3) urine test in men with previous negative biopsies: does free-to-total prostate-specific antigen ratio influence the performance of the PCA3 score in predicting positive biopsies? BJU Int. 106 (2010) 1143–1147.
[182] S.M. Aubin, J. Reid, M.J. Sarno, A. Blase, J. Aussie, H. Rittenhouse, R. Rittmaster, G.L. Andriole, J. Groskopf, PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: validation in the placebo arm of the dutasteride REDUCE trial, J. Urol. 184 (2010) 1947–1952.
[183] A. de la Taille, J. Irani, M. Graefen, F. Chun, T. de Reijke, P. Kil, P. Gontero, A. Mottaz, A. Haese, Clinical evaluation of the PCA3 assay in guiding initial biopsy decisions, J. Urol. 185 (2011) 2119–2125.
[184] A. Ruffion, M. Devonec, D. Champetier, M. Decaussin-Petrucci, C. Rodriguez-Lafrasse, P. Paparel, P. Perrin, V. Vlaeminck-Guillem, PCA3 and PCA3-based nomograms improve diagnostic accuracy in patients undergoing first prostate biopsy, Int. J. Mol. Sci. 14 (2013) 17767–17780.
[185] V. Scattoni, M. Lazzeri, G. Lughezzani, S. De Luca, R. Passera, E. Bollito, D. Randone, F. Abdollah, U. Capitanio, A. Larcher, G. Lista, G.M. Gadda, V. Bini, F. Montorsi, G. Guazzoni, Head-to-head comparison of prostate health index and urinary PCA3 for predicting cancer at initial or repeat biopsy, J. Urol. 190 (2013) 496–501.
[186] E.D. Crawford, K.O. Rove, E.J. Trabulsi, J. Qian, K.P. Drewnowska, J.C. Kaminetsky, T. K. Huisman, M.L. Bilowus, S.J. Freedman, W.L. Glover Jr., D.G. Bostwick, Diagnostic performance of PCA3 to detect prostate cancer in men with increased prostate specific antigen: a prospective study of 1962 cases, J. Urol. 188 (2012) 1726–1731.
[187] V. Vlaeminck-Guillem, M. Bandel, M. Cottancin, C. Rodriguez-Lafrasse, J.M. Bohbot, P. Sednaoui, Chronic prostatitis does not influence urinary PCA3 score, Prostate 72 (2012) 549–554.
[188] G.H. Leyten, E.A. Wierenga, J.P. Sedelaar, I.M. van Oort, J.J. Futterer, J.O. Barentsz, J.A. Schalken, P.F. Mulders, Value of PCA3 to predict biopsy outcome and its potential role in selecting patients for multiparametric MRI, Int. J. Mol. Sci. 14 (2013) 11347–11355.
[189] S. Perdona, D. Bruzzese, M. Ferro, R. Autorino, A. Marino, C. Mazzarella, G. Perruolo, M. Longo, R. Spinelli, G. Di Lorenzo, A. Oliva, M. De Sio, R. Damiano, V. Altieri, D. Terracciano, Prostate health index (phi) and prostate cancer antigen 3 (PCA3) significantly improve diagnostic accuracy in patients undergoing prostate biopsy, Prostate 73 (2013) 227–235.
[190] M. Salagierski, J.A. Schalken, Molecular diagnosis of prostate cancer: PCA3 and TMPRSS2:ERG gene fusion, J. Urol. 187 (2012) 795–801.
[191] C.E. Barbieri, C.H. Bangma, A. Bjartell, J.W. Catto, Z. Culig, H. Gronberg, J. Luo, T. Visakorpi, M.A. Rubin, The mutational landscape of prostate cancer, Eur. Urol. 64 (2013) 567–576.
[192] S.A. Tomlins, D.R. Rhodes, S. Perner, S.M. Dhanasekaran, R. Mehra, X.W. Sun, S. Varambally, X. Cao, J. Tchinda, R. Kuefer, C. Lee, J.E. Montie, R.B. Shah, K.J. Pienta, M.A. Rubin, A.M. Chinnaiyan, Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer, Science 310 (2005) 644–648.
[193] R. Esgueva, S. Perner, J. LaFargue, V. Scheble, C. Stephan, M. Lein, F.R. Fritzsche, M. Dietel, G. Kristiansen, M.A. Rubin, Prevalence of TMPRSS2-ERG and SLC45A3-ERG gene fusions in a large prostatectomy cohort, Mod. Pathol. 23 (2010) 539–546.
[194] S. Perner, F. Demichelis, R. Beroukhim, F.H. Schmidt, J.M. Mosquera, S. Setlur, J. Tchinda, S.A. Tomlins, M.D. Hofer, K.G. Pienta, R. Kuefer, R. Vessella, X.W. Sun, M. Meyerson, C. Lee, W.R. Sellers, A.M. Chinnaiyan, M.A. Rubin, TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer, Cancer Res. 66 (2006) 8337–8341.
[195] J. Groskopf, J. Siddiqui, S.M.J. Aubin, L. Sefton-Miller, J. Day, A. Blase, S. Varambally, J. Schalken, K. Sakamoto, Y. Fradet, H. Rittenhouse, A. Chinnaiyan, Feasibility and clinical utility of a TMPRSS2:ERG gene fusion urine test [Abstract], Eur. Urol. Suppl. 8 (2009) 195.
[196] A. Young, N. Palanisamy, J. Siddiqui, D.P. Wood, J.T. Wei, A.M. Chinnaiyan, L.P. Kunju, S.A. Tomlins, Correlation of urine TMPRSS2:ERG and PCA3 to ERG+ and total prostate cancer burden, Am. J. Clin. Pathol. 138 (2012) 685–696.
[197] S.S. Salami, F. Schmidt, B. Laxman, M.M. Regan, D.S. Rickman, D. Scherr, G. Bueti, J. Siddiqui, S.A. Tomlins, J.T. Wei, A.M. Chinnaiyan, M.A. Rubin, M.G. Sanda, Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer, Urol. Oncol. 31 (2013) 566–571.
[198] J.N. Cornu, G. Cancel-Tassin, C. Egrot, C. Gaffory, F. Haab, O. Cussenot, Urine TMPRSS2:ERG fusion transcript integrated with PCA3 score, genotyping, and biological features are correlated to the results of prostatic biopsies in men at risk of prostate cancer, Prostate 73 (2013) 242–249.

[199] G.H. Leyten, D. Hessels, S.A. Jannink, F.P. Smit, H. de Jong, E.B. Cornel, T.M. de Reijke, H. Vergunst, P. Kil, B.C. Knipscheer, I.M. van Oort, P.F. Mulders, C.A. Hulsbergen-van de Kaa, J.A. Schalken, Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer, Eur. Urol. 65 (2014) 534–542.

[200] S.A. Tomlins, S.M. Aubin, J. Siddiqui, R.J. Lonigro, L. Sefton-Miller, S. Miick, S. Williamsen, P. Hodge, J. Meinke, A. Blase, Y. Penabella, J.R. Day, R. Varambally, B. Han, D. Wood, L. Wang, M.G. Sanda, M.A. Rubin, D.R. Rhodes, B. Hollenbeck, K. Sakamoto, J.L. Silberstein, Y. Fradet, J.B. Amberson, S. Meyers, N. Palanisamy, H. Rittenhouse, J.T. Wei, J. Groskopf, A.M. Chinnaiyan, Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA, Sci. Transl. Med. 3 (2011) 94ra72.

[201] E. Dimitriadis, T. Kalogeropoulos, S. Velaeti, S. Sotiriou, E. Vassiliou, L. Fasoulis, V. Klapsas, M. Synesiou, A. Apostolaki, T. Trangas, N. Pandis, Study of genetic and epigenetic alterations in urine samples as diagnostic markers for prostate cancer, Anticancer Res. 33 (2013) 191–197.

[202] G. Robert, S. Jannink, F. Smit, T. Aalders, D. Hessels, R. Cremers, P.F. Mulders, J.A. Schalken, Rational basis for the combination of PCA3 and TMPRSS2:ERG gene fusion for prostate cancer diagnosis, Prostate 73 (2013) 113–120.

[203] M. Rigau, I. Ortega, M.C. Mir, C. Ballesteros, M. Garcia, M. Llaurado, E. Colas, N. Pedrola, M. Montes, T. Sequeiros, T. Ertekin, B. Majem, J. Planas, A. Ruiz, M. Abal, A. Sanchez, J. Morote, J. Reventos, A. Doll, A three-gene panel on urine increases PSA specificity in the detection of prostate cancer, Prostate 71 (2011) 1736–1745.

[204] D.L. Cao, D.W. Ye, H.L. Zhang, Y. Zhu, Y.X. Wang, X.D. Yao, A multiplex model of combining gene-based, protein-based, and metabolite-based with positive and negative markers in urine for the early diagnosis of prostate cancer, Prostate 71 (2011) 700–710.

[205] I. Katafigiotis, S.I. Tyritzis, K.G. Stravodimos, C. Alamanis, K. Pavlakis, A. Vlahou, M. Makridakis, A. Katafigioti, S.D. Garbis, C.A. Constantinides, Zinc alpha2-glycoprotein as a potential novel urine biomarker for the early diagnosis of prostate cancer, BJU Int. 110 (2012) E688–E693.

[206] T. Jamaspishvili, M. Kral, I. Khomeriki, V. Student, Z. Kolar, J. Bouchal, Urine markers in monitoring for prostate cancer, Prostate Cancer Prostatic Dis. 13 (2010) 12–19.

[207] M.J. Roobol, A. Haese, A. Bjartell, Tumour markers in prostate cancer III: biomarkers in urine, Acta Oncol. 50 (Suppl. 1) (2011) 85–89.

[208] D. Hessels, J.A. Schalken, Urinary biomarkers for prostate cancer: a review, Asian J. Androl. 15 (2013) 333–339.