Prostate-specific antigen (PSA) screening and new biomarkers for prostate cancer (PCa)

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ABSTRACT

PSA screening reduces PCa-mortality but the disadvantages overdiagnosis and overtreatment require multivariable risk-prediction tools to select appropriate treatment or active surveillance. This review explains the differences between the two largest screening trials and discusses the drawbacks of screening and its meta-analysis. The current American and European screening strategies are described.

Nonetheless, PSA is one of the most widely used tumor markers and strongly correlates with the risk of harboring PCa. However, while PSA has limitations for PCa detection with its low specificity there are several potential biomarkers presented in this review with utility for PCa currently being studied. 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. Another kallikrein panel, 4K, which includes KLK2 has recently shown promise in clinical

research studies but has not yet undergone formal validation studies. In urine, prostate cancer gene 3 (PCA3) has also been validated and approved by the FDA for its utility to detect PCa. The potential correlation of PCA3 with cancer aggressiveness requires more clinical studies. The detection of the fusion of androgen-regulated genes with genes of the regulatory transcription factors in tissue of ~50% of all PCa-patients is a milestone in PCa research. A combination of the urinary assays for TMPRSS2:ERG gene fusion and PCA3 shows an improved accuracy for PCa detection. Overall, the field of PCa biomarker discovery is very exciting and prospective.

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1. Prostate-specific antigen (PSA) and prostate cancer (PCa) screening

PSA-screening reduces PCa-specific mortality

The widespread and increasing use of PSA within the last 25 years has revealed PCa to be the most frequent malignancy in the Western world accounts for ~25% of all cancer cases in men [1, 2]. Since 2009, PSA-based screening for prostate cancer (PCa) has been heavily debated with clearly contrasting results of the two largest randomized screening studies. On one hand, the "European Randomized Study of Screening for Prostate Cancer" (ERSPC) with data on more than 162,000 men from 7 European countries found a PCa-specific mortality reduction of 20% [3] in the PSA screened group, which increased to 21% after a median follow-up of 11 years [4]. When the data is adjusted for nonattendance and PSA-contamination, the mortality risk reduction rises to 29-31% [5].

In marked contrast, the "prostate, lung, colorectal, and ovarian (PLCO) screening trial" with data from 76,693 American men, found no difference in the PCa-specific mortality after 7 years and also after 10 years of follow-up [6].

The reasons for these large differences and the drawbacks of general screening and several meta-analysis as well as the current screening strategies will be discussed in this review. The second key aspect in addition to screening in this review article is the evaluation of PSA and all PSA-based tumor markers and all currently available serum and urine biomarkers.

Differences between ERSPC and PLCO trial

First, the wide distribution of the PSA test in the U.S. resulted in a significant so-called PSA-contamination of the control group in the PLCO trial as at least 52% of the control group underwent at least one PSA test during the six years of screening. With a compliance rate of 85% in the screening group, the real difference in PSA testing was only 33%. However, it is more likely that within the PLCO screening trial actually only 15% of men in the control group never had a PSA test [7]. Thus, when 85% of men in the control group had a PSA test at least once in their life including 44% already before enter-

ing the study, the difference to the 85% of PSA-screened men is actual zero [8]. Thus, the PLCO trial became a comparison of frequent screening versus (somewhat) less frequent screening. Therefore, a mortality difference is very unlikely between PSA-screened and officially non-screened men in the PLCO trial. In the ERSPC study, the PSA contamination rate was much lower with 15% at the most [9]. The highest reported contamination from a single ERSPC center was 24% while other center specific contamination rates were below 10%. With 82% of all screen group participants screened at least once, the difference between screening and no screening in the ERSPC was 67% or at least 58%. This difference is ~2-fold in comparison to the PLCO (33%).

Second, for those screened men in the PLCO trial with positive tests (abnormal digital rectal examination (DRE) and/or PSA ≥4ng/ml) only 40.2% and 30.1% respectively, were biopsied [9]. The low biopsy rate indicates that two-thirds of men suspicious for PCa were not subsequently diagnosed. In the ERSPC, 85.8% of screening participants with positive tests (abnormal DRE and/or PSA ≥4ng/ml, changed 1996–1997 to PSA cutoff ≥3ng/ml without DRE) were in fact biopsied [3]. This rate is 2 to 3-fold higher as compared with the PLCO trial.

Third, there was no difference in stage distribution for all organ confined stages I and II between the screening arm (95.9%) and the control arm (94.4%) in the PLCO trial [6]. Also, the Gleason scores of ≤6 were not different between the screening (65.7%) and the control arm (60.3%). Since a PCa in such early stages normally does not show any symptoms, it is possible that the PSA-contamination in the control group was much higher than 52%. The stage distribution in the screening group in the ERSPC was 80.9% for stages I and II, while the control group had a significantly lower rate of the early stages with 58.9% [3]. Further, the proportions of men who had a less aggressive PCa with Gleason scores of ≤6 were 72.2% in the screening group and only 54.8% in the control group while a Gleason score ≥7 was detected in 27.8% in the screening group and in 45.2% in the control group [3]. These differences were predicted. Additionally, there was a relative reduction of 30% of detected metastatic PCa in the screening group [10].

Beside these above mentioned three important differences between both trials, the shorter follow-up of the PLCO trial as compared with the ERSPC [9] and the insufficient statistical power of the PLCO with its high PSA-contamination do also influence the PCa-specific mortality [8]. A reduced overall mortality should not be expected because the overall risk for men to die of PCa is reduced from 3% to 2.4% with PSA screening as shown from the ERSPC data [11].

All these characteristics of the PLCO trial with a narrow window of only 33% difference in screening between the two arms [9], the low biopsy rate, and the resulting identical stage distribution of the detected PCa in the screen and control arms [9], results in features that make the occurrence of a

difference in PCa-mortality unlikely even with a longer follow-up [9].

Drawbacks of screening

On the other hand, the 21% reduced PCa-specific mortality in the ERSPC that increased in single centers to 32% [12], 44% [13] or 51% with correction for nonattendance and contamination [14] has drawbacks with a significant overdiagnosis and detection of insignificant cancers. Overdiagnosis is a major problem for regular PSA screening and the risk to suffer from any PCa is 1.5-fold. The risk of a stage I PCa is almost 2-fold when summarizing data from 6 screening trials with almost 400,000 men [15]. While overdiagnosis itself may harm the patient in the way of a negative psychological effect, subsequent overtreatment can lead to incontinence, impotence and other clinical side effects. Data from the ERSPC show that 32-43% of low risk PCa may have avoided treatment [16]. In those cases, the active surveillance strategy is accepted as a non-treatment option for all low risk PCa-patients (reviewed in [17, 18]). Data from 439 patients initially screened and positively identified with PCa, showed no tumor progression in 86% of individuals after a 10 year follow-up [19].

While active surveillance becomes an increasingly popular management option it should be mentioned that the definition of those early disease stages only relies on biopsy results. An insignificant tumor on biopsy may become clinically significant in the final pathology of the prostatectomy specimen. Two studies on more than 12,000 men treated with radical prostatectomy showed that only 1/4 to 1/3 of tumors were still defined as insignificant on the final prostate pathology [20, 21]. Additionally, 1/5 to 1/3 also showed an upgrading from the biopsy to the final pathological result and ~10% had already extracapsular extension of the disease [20, 21]. This demonstrates that the proportion of men with an apparently insignificant PCa who actually have a clinical relevant tumor is not negligible [20, 21]. The topic of insignificant tumors has been already discussed elsewhere [22]. Finally, at long term follow-up after radical prostatectomy a biochemical recurrence occurs in up to 40% [23], indicating that these tumors were not insignificant but already in an advanced stage.

Problems of meta-analysis on PSA-screening

The differences between these two screening studies are substantial and it is questionable if data from the PLCO can be compared with the ERSPC data or used in meta-analysis [11]. Thus, it is not surprising that most meta-analysis incorporating the PLCO study and other screening studies with different clinical designs (reviewed in [24]) could not prove a lower PCa-specific mortality with PSA screening. Since 2010, at least 5 meta-analysis (including updates) have been published [15, 25-27], with almost all concluding no evidence of a PCa-specific mortality reduction. Only one meta-analysis found a 24% PCa-specific mortality reduction with PSA-screening using as exclusion criteria insufficient follow-up length, unacceptably high PSA-contamination in the control group or insufficient

participation in the screening group [27]. All other meta-analysis did not consider those important aspects. Exemplarily, the meta-analysis of Djulbegovic et al. [15] showed an inconsistency grade of 55% and should be therefore valued as questionable [24, 28]. It should be emphasized that a meta-analysis mostly based on studies with severe limitations cannot correctly answer the question of PSA-screening utility [29].

However, other screening studies without randomization (Tyrol study), with low numbers of patients and no PSA in the first two screening rounds (Norköping study), with a too short follow-up (French ERSPC) or with several methodological limitations (Quebec study) have been extensively reviewed elsewhere [15, 30] and will not be discussed here.

Current screening strategies

Despite strong discrepancies, the authors of the ERSPC and PLCO trial found shared conclusions for the future use of PSA in PCa screening [31]. PSA is able to predict PCa up to 30 years in advance [31]. Based on an initial PSA test (without age specification, but 40-45 years seems useful, at least before 60 years [32]) the frequency of follow-up PSA tests should be estimated depending on the individual PCa risk considering age, comorbidities, prostate volume, race and PCa family history [31]. With known PSA values, risk calculators can be used for biopsy indications [31].

Here, the current recommendation of the American Urological Association (AUA) and the European Urological Association (EAU) on PCa screening from 2013 should be mentioned [33, 34]. According to the American guidelines, PSA is not recommended for individuals below the age of 40 years or higher than 70 years. Regular biannual screening after careful counseling should be performed in men aged 55-69 years [33]. In Europe, a baseline PSA is recommended for men 40-45 years to initiate a risk-adapted follow-up approach with the purpose of reducing PCa-mortality and the incidence of advanced and metastatic PCa [34]. To prevent overdiagnosis and overtreatment, multivariable risk-prediction tools will be necessary [34]. This strategy seems to be a reasonably balanced approach so far.

The economically emphasized and widely distributed recommendation of the "US Preventive Services Task Force" completely abandoned PSA as screening tool [35] and has already been critically discussed elsewhere [11, 36].

Considering the above-mentioned points, we view the ERSPC results as reliable. A reduced PCa-specific mortality by more than 20% can be achieved. However, the likelihood of overdiagnosis is about 2-fold. PSA needs to be used in a more rational, strategic way and active surveillance should be included as a serious management option in appropriate patients.

2. Biology of PSA and its correlation with PCa

After the development of the first immunoassay for the PSA antigen in serum, the PSA test replaced

the PAP test and revolutionized the management of PCa. Biologically, PSA is responsible for semen liquefaction and secreted into the seminal plasma but a retrograde release of PSA into the blood-stream is a rare event in healthy men (reviewed in [37]). An excessive escape of PSA into the blood circulation only occurs in cases of destruction of the basement membrane of prostate epithelial cells. Although an increased PSA can also be caused by benign prostate diseases, such as benign prostate hyperplasia (BPH) or prostatitis, there is a strong correlation of serum PSA with the incidence of PCa [37]. Thus, increased PSA levels indicate pathologies of the prostate gland including PCa, but PSA is not cancer-specific. In addition to the relationship of an elevated PSA with a higher PCa risk, PSA can predict the occurrence of PCa several years in advance as already mentioned [37]. Furthermore, PSA can predict death from PCa with up to 25 years in advance [38, 39]. The risk to die from metastatic PCa is as high as 44% for men aged 45 to 55 years when their PSA is within the 10th percentile as compared with those men with a PSA below the median with a risk of <0.3% [39].

3. Efforts to overcome PSA limitations

While PSA is the key parameter for the management of a known PCa, there are decisive limitations for diagnosing PCa. As mentioned, benign prostate diseases as well as prostate manipulations such as bicycling, digital rectal exam (DRE), biopsy, catheterization or ejaculation can also cause at least temporary elevated PSA serum concentrations [40]. This leads to low specificity if a single PSA measurement is used to predict PCa, especially in the PSA "grey zone" of 2-10ng/ml [40]. Avoiding factors such as bicycling, DRE or ejaculation a few days before a PSA blood draw may facilitate interpretation of results. In addition, a biological variation of the PSA value up to 20-30% [41] should be considered. A simple repeat measurement of PSA can significantly reduce the number of prostate biopsies [42] but 60-80% of all biopsies are still unnecessary. The traditional PSA cutoff of 4ng/ml is no longer valid because the PCa detection rate at the 2-4ng/ml range [43] is comparable to the 4-10ng/ml range in the PSA screening environment today [44]. Further, differences between PSA assays additionally with or without WHO calibration may complicate the interpretation of results [45, 46].

To increase the specificity of PSA, different parameters have been developed like PSA density (ratio of PSA to prostate volume), PSA velocity (change of PSA over a time period) or age-/race-specific reference ranges [40]. All these PSA based parameters have been only partially successful. PSA density is perhaps the single most specific parameter but requires an ultrasound procedure to obtain an accurate assessment of prostate size.

4. PSA based serum markers

PSA complexes with proteinase inhibitors

In the early 1990s two independent groups found PSA to exist in different molecular forms [47, 48]. Ap-

proximately 65-95% of PSA is bound to alpha-1-antichymotrypsin (PSA-ACT) while the remaining PSA circulates as free PSA (fPSA). PSA-ACT is higher in PCa-patients compared with non-PCa-patients [48]. The enzymatically-active form of PSA is rapidly and irreversibly complexed with prostate inhibitors while inactive PSA (free PSA) is not complexed [49]. PSA also complexes with alpha2-macroglobulin (A2M), which is not measurable with the current assays. The measurement of PSA-A2M needs rather complicated methods [50]. A very small amount of PSA is also complexed with the protease inhibitor alpha1-protease inhibitor (API). The very small amounts of API to total PSA (tPSA) are analytically challenging [51] so that both the A2M-PSA complex and API-PSA complex assays have never become commercially available. Current PSA immunoassays measure free and complexed PSA which is sometimes referred to as total PSA.

Using a blocking antibody against fPSA, all complexed PSA (cPSA) can be also measured. The cPSA only reaches comparable results to the ratio of fPSA to tPSA (f/tPSA ratio or percent free PSA, %fPSA) when also used as ratio to tPSA, but not as a single parameter [52]. Since the tPSA is the sum of ACT-PSA and fPSA the ratios of cPSA to tPSA should be equal to fPSA to tPSA in a clinical correlation. The fPSA to tPSA ratio was used earlier than cPSA to tPSA ratio. Therefore the vast majority of clinical utility studies on molecular forms of PSA have been published on %fPSA.

Clinical relevance of %fPSA

Since the middle of the 1990s the %fPSA has become a clinically relevant parameter to improve specificity of PSA alone [53]. This has been confirmed (reviewed in [54]). A meta-analysis on %fPSA found an area under the ROC curve (AUC) of 0.68 for more than 2800 patients within the tPSA "grey zone" of 4-10ng/ml [55]. But the authors concluded that %fPSA can only be a useful adjunct to PSA-based screening when reaching extreme values such as <7% [55]. When using high %fPSA cut-offs, the number of unnecessary biopsies could be reduced by ~10-20%. However, for a more accurate interpretation, factors such as prostate volume, prostatitis, or prostatic intraepithelial neoplasia should be considered (reviewed in [52]). Currently, %fPSA is used within multivariable models such as artificial neural networks (ANN) or logistic regression (LR) based nomograms to predict the PCa risk in a subsequent prostate biopsy (reviewed in [56]). Table 1 (modified from ref. [56]) shows the improvement of %fPSA and ANN or LR models compared with tPSA. Regardless of the different assays for tPSA and fPSA [57] and the different PSA ranges investigated, enhanced specificities by using %fPSA within ANN or LR models were observed. However, only some models are freely online available [57, 58] as illustrated in Figures 1 and 2.

Subforms of free PSA

Beginning in 2000, researchers focused to define subforms of fPSA in search for ways to further enhance the specificity of %fPSA. The free PSA became more complex [59]. Figure 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. The bPSA could potentially be used as marker for BPH, but was unable to distinguish between BPH and PCa [60, 61]. But within a multivariable model, bPSA improved specificity of %fPSA by ~15% [61].

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) [62]. Although iPSA could distinguish between PCa and BPH, its further use has been limited since a commercial assay is lacking. A lab-based test may now be available as a panel termed 4K. This panel combines tPSA, fPSA, iPSA and the human glandular kallikrein 2 (KLK2) and showed a high predictive accuracy [63, 64].

Another subform, proPSA is termed [-7]proPSA and contains a seven amino acid N-terminal pro-leader peptide in this native form, which is rapidly truncated by proteolytic cleavage to [-4]proPSA, or [-2] proPSA. The proPSA derivative [-2]proPSA cannot be cleaved to form enzymatically-active PSA and accumulates in the prostate cancer regions of the prostate. A research assay measuring the [-7, -5] proPSA was of limited usefulness and has subsequently not been commercialized (reviewed in [52]).

Only the [-2]proPSA [65] and especially the commercial and FDA-approved [-2]proPSA [66, 67] showed the expected further improvement in specificity over %fPSA. Since 2010 the Prostate health index (phi) (calculated as: [-2]proPSA / fPSA * √PSA) has been used to discriminate between PCa and non-PCa [68]. These data on phi have been confirmed in large multicenter cohorts and it further seems that phi may preferentially detect aggressive PCa [66, 67, 69, 70].

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 4-10ng/ml and negative DRE. A comprehensive review summarizes all aspects on different proPSA forms as well as the cost-effectiveness of phi [71]. 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 biopsies [71].

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 tPSA range of 2-10ng/ml [72]. At 90% sensitivity a pooled specificity of ~32% for phi and %[-2]proPSA was found; both parameters were superior to tPSA and %fPSA. Table 2 provides data on all available studies using phi with at least 200 biopsy proven patients.

Increasing phi values were associated with an increased probability of detecting Gleason ≥7 PCa [66, 67]. Two studies with more than 2,200 men independently found that the relative risk of any PCa is

3.6-fold [67] to 4.7-fold [66] higher in those men with phi values in the highest as compared with the lowest quartile. The risk of a Gleason ≥7 PCa increases 1.6-fold with phi values the highest quartile [66]. Phi had also significantly higher median values in aggressive PCa and the proportion of Gleason ≥7 PCa increased with the phi score [67].

However, when using phi within multivariable models, the AUC-gain was very modest or not visible [67, 73]. As reviewed [74], the inclusion of new biomarkers such as urinary prostate cancer antigen 3 (PCA3) and [-2]proPSA in risk calculators amounted only to a marginal improvement in the accuracy of these prediction tools. Despite this, phi shows overall promising data, especially when focused to detect aggressive PCa.

5. Other prostate cancer serum marker

The kallikreins

Beside the pancreatic/renal kallikrein KLK1, KLK2 and KLK3 which is widely known as PSA, 12 new members of the human kallikrein family have been characterized [75]. The human kallikrein genes are named *KLK1* to *KLK15* and they encode for the proteins KLK1 to KLK15.

KLK2 can convert proPSA to active PSA (reviewed in [52]) and has been investigated extensively [75]. However, early promising data could not be confirmed (reviewed in [52] and [54]) and KLK2 has not been transferred into a commercial assay.

Beside KLK2 and PSA, at least 6 other kallikreins (*KLK4, KLK10-13 and KLK15*) are also expressed in relatively high amounts in prostate tissue [75] but again, no commercial immunoassay is available. Only KLK11 showed promising values but data have not been confirmed independently (reviewed in [52] and [54]). Reviews on kallikreins have been published elsewhere [75, 76].

Other serum markers

Details on several markers like caveolin, IGF, PSP94, macrophage inhibitory cytokine 1, cytokine macrophage migration inhibitory factor, the calcium-binding proteins S100A8 and S100A9 that have never reached clinical significance or at least assay commercialization have been already reviewed [77].

The extracellular matrix protein Spondin-2 [78], and Galectin-3, a tumor-associated protein [79], 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) [78]. The galectin-3 levels were in contrast only compared in the sera of metastatic PCa-patients with non-cancer patients [79].

Sarcosine in serum

In the above mentioned study on Spondin-2 [78], sarcosine showed limited success. Others found an increased PCa risk and a further increased risk for aggressive PCa (odds ratio 1.44) with increasing

sarcosine levels [80]. In contrast, another large study found high sarcosine and glycine concentrations to be associated with a reduced PCa risk of borderline significance (odds ratio 0.86) [81]. Other studies on sarcosine in serum and plasma with smaller numbers of patients also showed inherent data [82-84]. Interestingly, first data on sarcosine have been published in urine.

6. Urine markers

Sarcosine

Sreekumar et al. [85] found sarcosine to be significantly higher in urine sediments and supernatants in PCa as compared with men without PCa [85]. In 53 men, the AUC for sarcosine (0.69) was significantly higher than the AUC of PSA (0.53) at PSA levels of 2-10ng/ml [85]. In contrast, another study in 139 men found significantly lower sarcosine values in PCa-patients compared with non-PCa-patients and no difference between healthy men and PCa-patients [86]. Also, %fPSA (AUC: 0.81) had a significantly larger AUC than sarcosine (0.63), and PSA (0.64) was equal to sarcosine [86]. Sarcosine was measured with a commercial amino acid assay and values were normalized to urine creatinine [86]. There was a strong correlation (r_s=0.86) between the sarcosine and creatinine showing that the occurrence of sarcosine in urine is due to renal excretion [86]. Sarcosine is not specific to prostate tissue nor is it related to tumor aggressiveness or recurrence, which is in contrast to PCA3, which is prostate-specific and not found elsewhere. Therefore it is unlikely that sarcosine is suitable as a marker for PCa detection. Further details on sarcosine have been reviewed recently [87].

PCA3

PCA3 is a noncoding messenger RNA (mRNA) and is 66-fold overexpressed in PCa tissue. A molecular assay for PCA3 was introduced in 2006 [88]. In 2012, this assay was FDA-approved to aid in the decision for repeat biopsy in men ≥50 years (reviewed in [89]).

Two independent multicenter studies found excellent clinical value of the PCA3 assay in men with previous negative biopsies [90] and with first and repeat biopsies [91]. Haese et al. [90] found PCA3 to be better than %fPSA and that PCA3 was independent of prostate volume, age and tPSA. The PCa likelihood increased with the PCA3 score. However, the PCa detection rate was only 47% in those patients with PCA3 scores >100 [90]. This problem has been reported in several other studies (reviewed in [89]) proving a low sensitivity with a PCA3 cutoff of 100.

Nonetheless, several studies have proven the clinical value of PCA3 to improve specificity over PSA and %fPSA (reviewed in [89]). Table 3 provides data on studies with at least 200 patients. With exception of two studies (AUC 0.59 and 0.83), the AUCs for PCA3 are ~0.7.

However, PCA3 is not capable to replace PSA as a first-line test in clinical practice due to the lack of an appropriate cut-off level with acceptable performance characteristics. But addition of PCA3 to risk

assessment tools leads to an increase of 4.5-7.1% in predictive capability [92, 93].

In contrast to PSA, PCA3 is not influenced by prostate volume, prostatitis or medication with 5-alpha-reductase inhibitors (reviewed in [89]). Regardless of its complicated measurement procedure, relative high costs (~300 Euros), and lower sensitivity than PSA PCA3 has clearly shown its clinical value. The potential correlation of PCA3 with tumor volume and cancer aggressiveness has shown conflicting results in several studies and needs to be clarified.

TMPRSS-2

The detection of gene fusions involving the androgen regulated TMPRSS2 and ETS transcription factor genes in PCa was a research-milestone [94]. Approximately 50% of all PCa-patients do have the TMPRSS2 fusion with the ETS family member that is regarded as a key PCa oncogene [94, 95]. Based on these important findings in PCa tissue, a urinary assay using the same format as PCA3 has been developed [96]. TMPRSS2:ERG in PCa tissue and in urine showed a strong correlation demonstrating a high tumor specificity of this marker. 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 [97]. So far, only one study reported separate data on the TMPRSS2:ERG urine assay in a larger (n=246) and more balanced cohort [98]. ROC data showed a significant lower AUC for TMPRSS2:ERG (0.63) than for PCA3 (0.74) but both had no difference to phi (AUC 0.68) [98]. All other studies only reported AUCs on PCA3 and TMPRSS2:ERG together without separate evaluation of TMPRSS2:ERG [99, 100].

The use of TMPRSS2:ERG and PCA3 in PCa risk calculators has been published [99, 100]. The TMPRSS2:ERG had independent additional predictive value to PCA3 and to the ERSPC risk calculator parameters for predicting PCa [99]. TMPRSS2:ERG had prognostic value, whereas PCA3 did not [99]. Urinary TMPRSS2:ERG was further associated with clinically significant PCa at biopsy and prostatectomy [100].

It was postulated, that there is a rational basis for the need to combine PCA3 and TMPRSS2:ERG gene fusion for PCa diagnosis [101]. Based on tissue expression, it was visible that most false-negative results of PCA3 were corrected by TMPRSS2:ERG and that the combination of both markers would be capable to improve sensitivity [101].

Conclusion on urine markers

Detecting PCa in urine is technically feasible, as demonstrated by numerous studies, but few markers have been validated in multiple large sample sets [102]. There are several new markers like zinc alpha2-glycoprotein, thiosulfate or combinations of markers measured in multiplex models or gene panels that are only reported by one group so far. However, preanalytical conditions in urine are more difficult than in serum and the process of urine collection is subject to variability, which may result in conflicting clinical results [102].

Details on further urine marker have been published elsewhere [102, 103]. However, advanced clinical studies have identified only PCA3 and TMPRSS2:ERG fusion transcripts as promising RNA markers for cancer detection and possibly prognosis [102].

Summary

PSA screening reduces PCa-mortality as shown by the largest screening trial so far, the ERSPC. Other screening trials and meta-analysis from these trials with severe drawbacks should be interpreted cautiously. However, disadvantages of regular screening, namely overdiagnosis and overtreatment can be diminished with selective strategies including active surveillance. The most balanced screening guideline, the European EAU screening guideline, recommends a baseline PSA for men with 40-45 years to initiate a risk-adapted follow-up approach to reduce PCa-mortality and the incidence of advanced and metastatic PCa.

PSA as one of the most widely used tumor markers strongly correlates with the risk of harboring from PCa. This risk is already visible up to 20-30 years in advance but PSA has severe limitations for PCa detection with its low specificity. The FDA-approved and currently best serum parameter phi shows improved specificity over %fPSA and PSA. The best parameter in urine, the FDA-approved PCA3 has also been proven its utility in the PCa detection but correlation with aggressiveness and low sensitivity at high values have to be re-examined. While the detection of TMPRSS2:ERG gene fusion was one research milestone, the urinary assay for TMPRSS2:ERG only shows the expected improved accuracy for PCa detection in combination with PCA3.

Taken together, risk-adapted PSA screening and diagnosing as well as appropriate use of the FDA-approved biomarkers is the most likely scenario in the near future. New techniques such as genomics, proteomics or metabolomics as well as improved imaging devices (multiparametric-MRI) and the simultaneous use of all parameters preferentially within multivariable models may further enhance the accuracy of PCa diagnosis within the next years.

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Table 1. **Examples for multivariate models using %fPSA for diagnosis of PCa (1998-2004)**

First Author [Ref.] (n of pts.; % of PCa)	Year	Scree-ning	Model (ranking)	PSA assays (company)	tPSA range (ng/ml)	contributing factors (if numbered, by value)	AUC	Specificity at 95% sensitivity
Carlson (n=3773; 33% PCa)	1998	no	LR	Tosoh (Dianon)	4-20	1.%fPSA, 2.age 3.tPSA	n.a.	34 (LR) 23 (%fPSA)
Virtanen (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.

(Table 1 cont'd) **Table 1. Examples for multivariate models using %fPSA for diagnosis of PCa (1998-2004)**

First Author [Ref.] (n of pts.; % of PCa)	Year	Scree-ning	Model (ranking)	PSA assays (company)	tPSA range (ng/ml)	contributing factors (if numbered, by value)	AUC	Specificity at 95% sensitivity
Finne [58] (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 (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 (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 (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 (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 (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)
Sokoll [70] (n=566; 43% PCa)	2010	not available	0.79 (LR model)	80	45			

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

Table 2. Selected studies with more than 200 subjects on Phi (2010-2013)

First author [Ref.] (n of pts.; % of PCa)	Year	Phi cutoff	AUC	Sensitivity	Specificity
Sokoll [70] (n=566; 43% PCa)	2010	not available	0.79 (LR model)	80	45
Jansen [68] (n=756; 50% PCa)	2010 2 cohorts	not available	0.75 (0.71)	90	31
Liang (n=250+250; 50% PCa, matched)	2011	36.45 (at 90% spec.)	0.73	42	90
Guazzoni (n=268; 40% PCa)	2011	48.5 (at 90% spec.) Hybr. calibr.	0.76	43	90
Catalona [66] (n=721; 17% PCa)	2011	21.3 (24.1) Hybr. calibr.	0.70	95 (90)	16 (26)
Loeb (see also [66]) (n=721; 17% PCa)	2013	24.3 (27.9) WHO calibr.	0.70	95 (90)	16 (27)
Lazzeri (n=222; 32% PCa)	2012	28.8 Hybr. calibr.	0.67	90	25
Stephan [67] (n=1362; 49% PCa)	2013	31 (24)	0.74	95 (90)	15 (35)
***Stephan** [98] (n=246; 45% PCa)	2013	27.5	0.68	90	21
***Ferro** (n=300; 36% PCa)	2013	31.6	0.77	90	40
Ito (n=239; 22% PCa)	2013	23.9 (24.9) Hybr. calibr.	0.72	95 (90)	28 (33)
Lazzeri [69] (n=646; 40% PCa)	2013	27.6 41.5 61.7	0.67	90 63 25	19 62 90
***Scattoni** (n=211; 33% PCa)	2013	28.3 (30.6) 24.1 (35.5)	0.70 all 0.69 1st bx 0.72 2nd bx	90 (80) 90 (80)	16-34 7-47
Ng (n=230; 9% PCa)	2013	26.5 Hybr. calibr.	0.78	90	50

*also PCA3 values available

Abbreviations: AUC: area under the (ROC) curve; bx: biopsy; Hybr. calibr.: Hybritech calibration (for PSA & fPSA); n.a.: not available; WHO calibr.: calculated (not measured) as WHO calibrated

Table 3. Selected studies with more than 200 subjects on PCA3 (2007-2013)

First author [Ref.](n of pts.; % of PCa)	Year	PCA3 cutoff	AUC	Sensitivity	Specificity
Marks (n=226; 27% PCa)	2007	35	0.68	58	72
Haese [90](n=463; 28% PCa)	2008	35	0.66	47	72
Deras [91](n=570; 36% PCa)	2008	35	0.69	54	74
Ankerst (n=443; 28% PCa)	2008	25	0.665	63	60
Chun [92] (n=809; 39% PCa)	2009	17	0.68	81	45
Hessels (n=336; 40% PCa)	2010	35	0.72	61	74
Auprich (n=621; 41% PCa)	2010	17 (24, 35)	0.73-0.75	88	45
Roobol (n=721; 17% PCa)	2010	35	0.635	68	56
Ploussard (n=301; 24% PCa)	2010	35, (25, 30)	0.69	44-59	67-79
Aubin (n=1072; 18% PCa)	2010	35	0.69	48	79
De la Taille (n=516; 40% PCa)	2011	35	0.76	64	76
Perdona (n=218; 33.5% PCa)	2011	51	0.83	70	81
Bollito (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 (n=1913; 42% PCa)	2012	10 (25, 35)	0.71	86.5	37
Stephan [98](n=246; 45% PCa)	2013	28	0.74	73	64
Hansen [93](n=692; 46% PCa)	2013	21	0.74	79	59
Scattoni (n=211; 33% PCa)	2013	16.5 (13.5, 23.5)	0.59	80 (90)	16-34
Tombal (n=1024; 18% PCa)	2013	20	n.a.	87	55
Gittelman (n=466; 22% PCa)	2013	25	0.71	77.5	57
Ferro (n=300; 36% PCa)	2013	22	0.73	90	40
Goode (n=456; 19% PCa)	2013	35	0.73	62	75
Ruffion (n=601; 46% PCa)	2013	35	0.74	63	72

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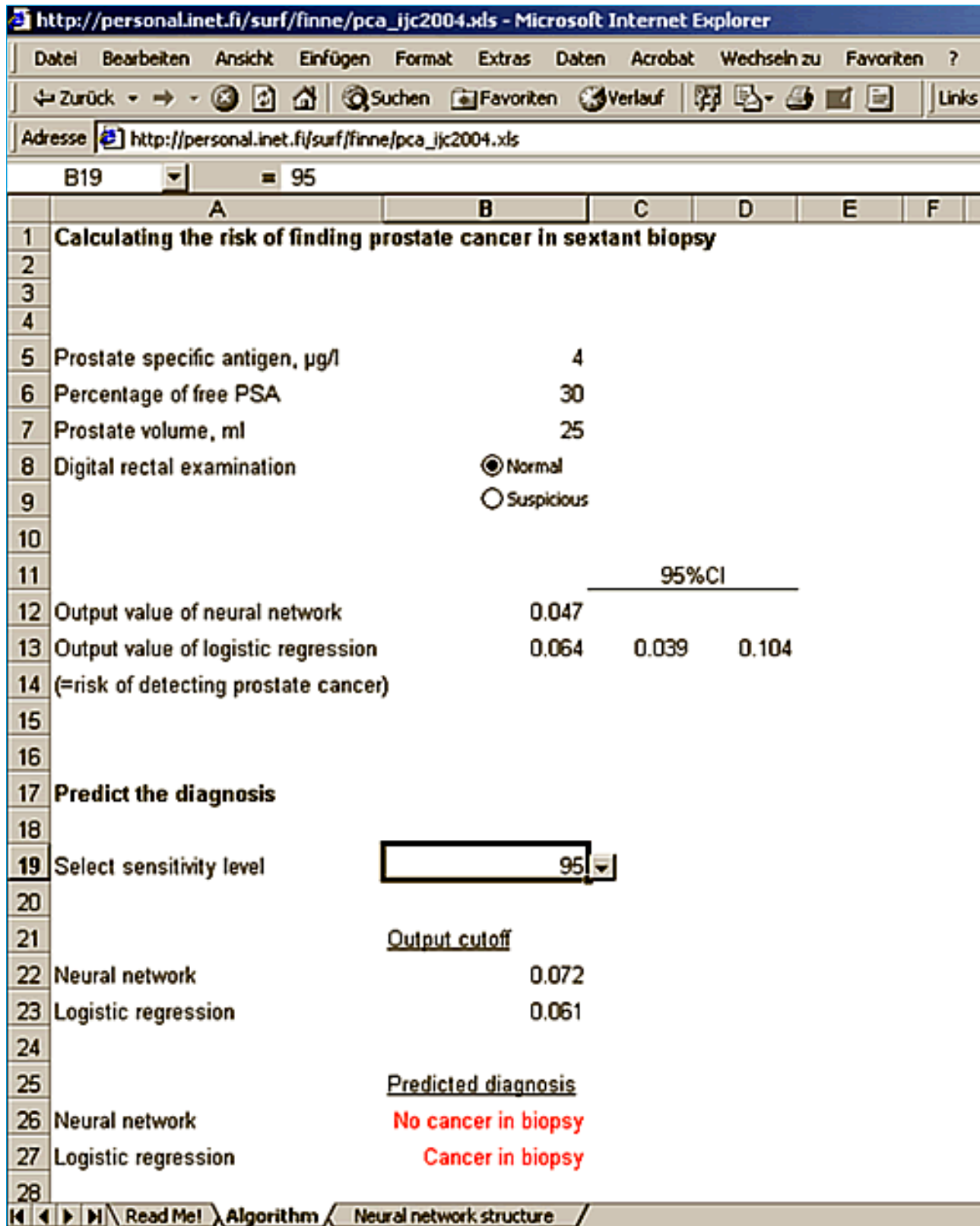on ANN and LR at the 95% sensitivity level.

Fig. 2. Program "ProstataClass" version 2008 for 5 different PSA assays at http://urologie.charite.de and the link: "ProstataClass". Provided example of the ANN output (only available in German) indicating "Risiko" (risk)" at the 95% sensitivity level.
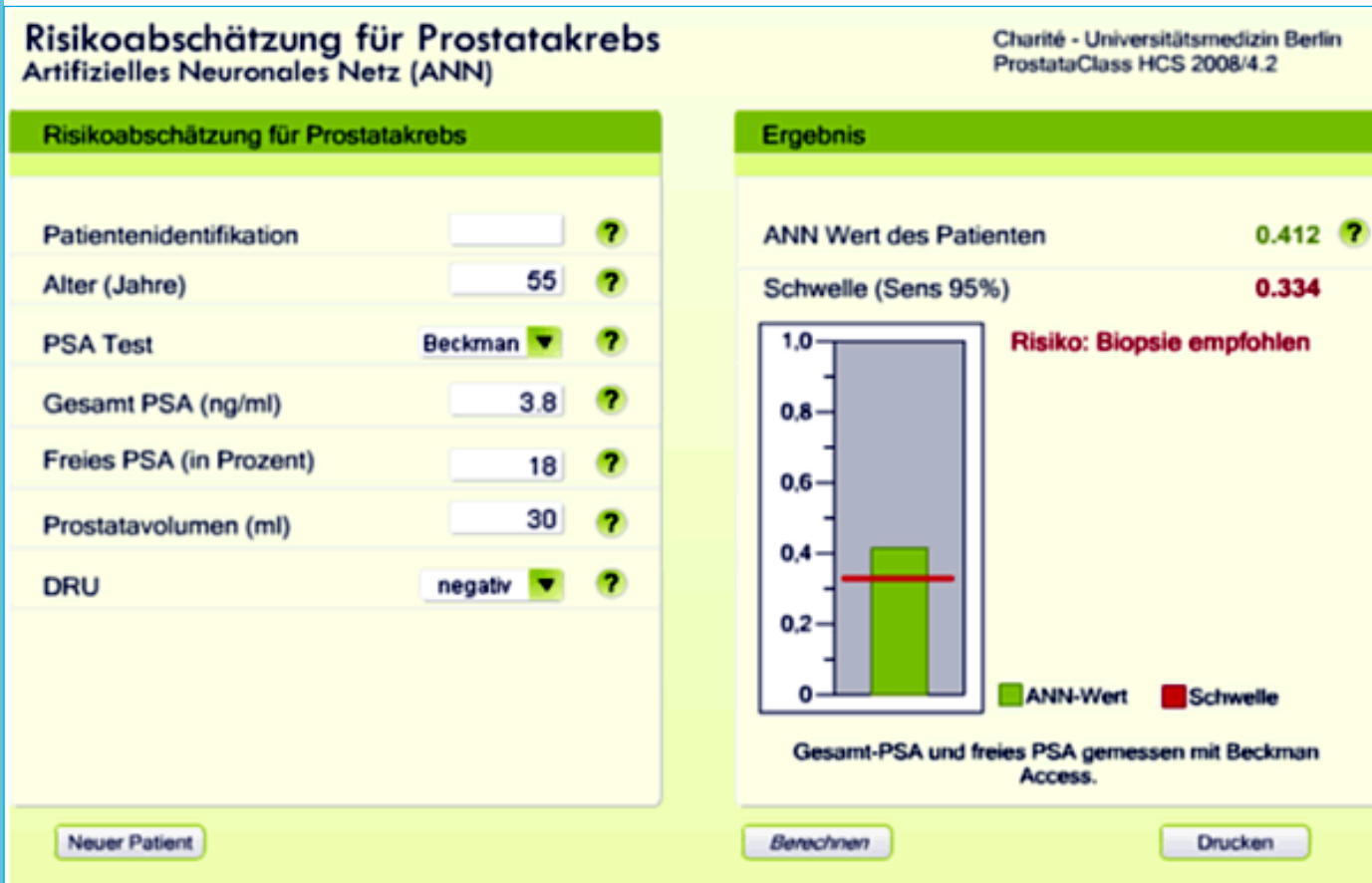

Fig. 3. Molecular forms of PSA and the prostate health index phi including the respective times of detection.