Urinary Prostate Cancer Antigen 3 as a Tumour Marker: Biochemical and Clinical Aspects

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Abstract

Due to low specificity of Prostate-Specific Antigen (PSA) we face a certain risk of overdiagnosis and overtreatment of Prostate Cancer (PCa). The benefits and harms of PSA-screening are controversially discussed. To overcome this weakness of PSA novel PCa biomarkers and detection tools are required.

The urine-based biomarker Prostate Cancer Antigen 3 (PCA3) has been shown to be highly PCa-specific. Application of PCA3 was tested in the diagnostic setting and staging. Several studies pointed out the additional value of PCA3 for further stratification of men selected for biopsy (BX) based on an elevated PSA and/or an abnormal digital rectal examination (DRE). Its combined use with established clinical risk factors for positive prostate BX, particularly within nomograms or risk calculators, may represent a valid and helpful aid for clinicians in patient counselling and BX indication confirmation.

When it comes to prediction of favourable or unfavourable histopathological features, respectively, such as tumour volume or PCa significance, PCA3's value remains controversial. Based on relatively small patient numbers, PCA3 has been identified to independently predict small-volume and insignificant PCa. However, in other studies PCA3 was not associated with advanced disease and its ability of predicting PCa aggressiveness in men undergoing radical prostatectomy is limited.

PCA3's value may be best given for BX outcome prediction. Finally, the implementation of the PCA3 promoter in developing new highly PCa-specific gene therapies represents a promising perspective in the near future.

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Keywords

5a-reductase inhibitor • AMACR • Circulating tumour cells • Diagnosis • Gene therapy • Gleason score • GOLM1 • PCA3 • PCA3 nomogram • Performance characteristics • Prostate biopsy • Prostate cancer • Prostate cancer antigen 3 score • PSA • SPINK1 • Transmembrane-serine protease • Urine analysis

Abbreviations

%fPSA	Percent-free PSA
AMACR	A-methylacyl-coenzyme racemase
AS	Active surveillance
AUC	Area under the curve
BPH	Benign prostate hyperplasia
CE	Conformiteé européenne
CTC	Circulating tumour cell
DHT	Dihydrotestosterone
DRE	Digital rectal examination
EAU	European Association of Urology
ECE	Extracapsular extension
ERG	V-ets erythroblastosis virus E26 oncogene
ERSPC	European Randomised Study of Screening for Prostate Cancer
FDA	US Food and Drug Administration
GOLM1	Golgi membrane protein 1
GS	Gleason score
IL	Interleukin
mRNA	Messenger Ribonucleic Acid
PCa	Prostate cancer
PCA3	Prostate Cancer Antigen 3
PCPT-RC	Prostate Cancer Prevention Trial risk calculator
PSA	Prostate Specific Antigen
REDUCE	Reduction by Dutasteride of Prostate Cancer Events – trial
RP	Radical prostatectomy
RT-PCR	Reverse transcriptase polymerase chain reaction
SPINK1	Serine peptidase inhibitor Kazal type 1
TMPRSS2	Transmembrane-serine protease gene
TV	Tumour volume

17.1 Introduction

Prostate Cancer (PCa) represents the most common disease affecting men, with 238.590 estimated new cases diagnosed in 2013 in the United States (US) [1]. Due to the implementation of total serum Prostate-Specific Antigen (tPSA) in clinical practice for PCa screening and detection, incidence rates of PCa have increased dramatically over time [2]. The rationale behind PCa screening is to reduce the prevalence of advanced disease and PCa-related mortality. However, due to the heterogeneity of PCa cancer subtypes most patients have slow-growing tumours and have minimal risk of dying from the disease [3–5]. On the other hand, there are aggressive tumours resulting in significant morbidity and death. During the decision-making process of biopsy (BX) indication for PCa diagnosis or active PCa treatment after histologically confirmed PCa, the treating physician as well as the patient have to be aware of this dilemma. The challenge in managing clinically localized disease is to distinguish between men with aggressive cancers who would truly need immediate therapy, and those with less aggressive disease who can be safely managed by e.g. active surveillance (AS). As a consequence efforts are made to improve PCa detection performance, risk assessment and surveillance. It is known that PSA testing shows limited specificity mainly in lower PSA ranges [3]. However, increased PSA levels do not reflect PCa exclusively, but also indicate benign prostate enlargement (BPH) and/or inflammatory reactions [6]. In this setting, novel biomarkers represent a promising component to increase the specificity of PCa detection [7]. One of these novel biomarkers is Prostate Cancer Antigen 3 (PCA3). PCA3 messenger Ribonucleic Acid (mRNA) is

highly overexpressed in prostatic tumours [8] and represents a urine-based biomarker that has been widely examined and shown to keep up to its promise. When the third-generation of urinary PCA3 assay (Progensa® PCA3; Hologic Gen-Probe Inc., Bedford, MA, USA) [9] attained Conformiteé européenne (CE) approval in 2006, several clinical studies were conducted to evaluate PCA3 as a novel diagnostic marker, to counsel patients or to confirm BX indications and/or to rule out aggressive cancer at BX, respectively. Beside BX endpoints, the clinical staging significance of preoperative urinary PCA3 was assessed to identify respectively favourable and/or unfavourable histopathological features, such as small tumour volume/insignificant PCa vs. locally advanced disease and aggressive disease. Based on promising findings from previous studies, the novel marker was further evaluated in its ability as a first-line diagnostic test in prescreened men [10–19]. In these studies, specificities range between 71 % and 93 % for prediction of PCa at BX in men with elevated PSA levels, whereas the corresponding sensitivities range from 47 % to 75 % when PCA3 is used in isolation. The observed differences are due to the different PCA3 cut-offs that are used. A plethora of PCA3 cut-offs have been tested, still leaving the question of the "best" cut-off unsolved, even though the U.S. Food and Drug Administration (FDA) has recently approved a PCA3 score cut-off of 25 as justification for a repeat BX [20]. In contrast to the PCA3 score cut-off of 25, Auprich et al. conclude that PCA3 may be most clinically relevant in the repeat BX setting, when using a cut-off of 35 to confirm repeat prostate BX indication [21]. However, so far no cut-off seems to provide a reasonable trade-off for sensitivity and specificity when PCA3 is used in isolation. Furthermore, it has to be considered that biomarkers should ideally be used as a continuous variable instead of using cut-offs, since risk levels are not truly discrete but represent a continuum of risk [22, 23]. Nonetheless, according to the European Association of Urology (EAU) Guidelines 2011 [24] the use of PCA3 in the detection setting of PCa is not any more classified as experimental. Integrated in novel BX nomograms, PCA3 can be a useful aid for patient counselling and BX indication confirmation, and it may also be used to determine whether a men needs a repeat BX after an initially negative BX outcome (evidence level 2A).

Concerning usefulness of PCA3 in men undergoing active surveillance (AS) so far no evidence has been presented [21].

Beyond these clinical implications, further research was also directed at evaluating its potential use in combination with other new biomarkers, and as a novel target for PCa therapy.

17.2 Prostate Cancer Antigen 3

17.2.1 History

By comparing PCa tissue with non-malignant prostatic tissue, Bussemakers et al. firstly identified the DD3 (later called *PCA3*) gene in 1999 (Fig. 17.1), functioning as non-coding RNA and mapping to chromosome 9q21–22. Using a reverse transcriptase polymerase chain reaction (RT-PCR) method, they detected that PCA3 was overexpressed in cancerous tissue and low expressed in benign prostatic tissue and not measurable in the normal tissue of numerous organs such as the testis, bladder, kidney, seminal vesicles, brain and lung. PCR3 is highly prostate specific and was overexpressed in 95 % of tumour lesions, but in only 1 of 7 human PCa cell lines (lymph node carcinoma of the prostate) and in none of 18 non-malignant prostate samples [8]. A multitude of studies further implicated significantly higher PCR3-mRNA expression in prostatic tumours in comparison to non-malignant prostatic tissue [19, 21–23, 25] (Fig. 17.2). These findings promoted the idea of developing a PCA3 diagnostic test.

17.2.2 Urine Analysis

There are several urinary assays measuring PCA3 mRNA, which is highly upregulated in neoplastic prostate tissue [9, 26, 27]. The assays

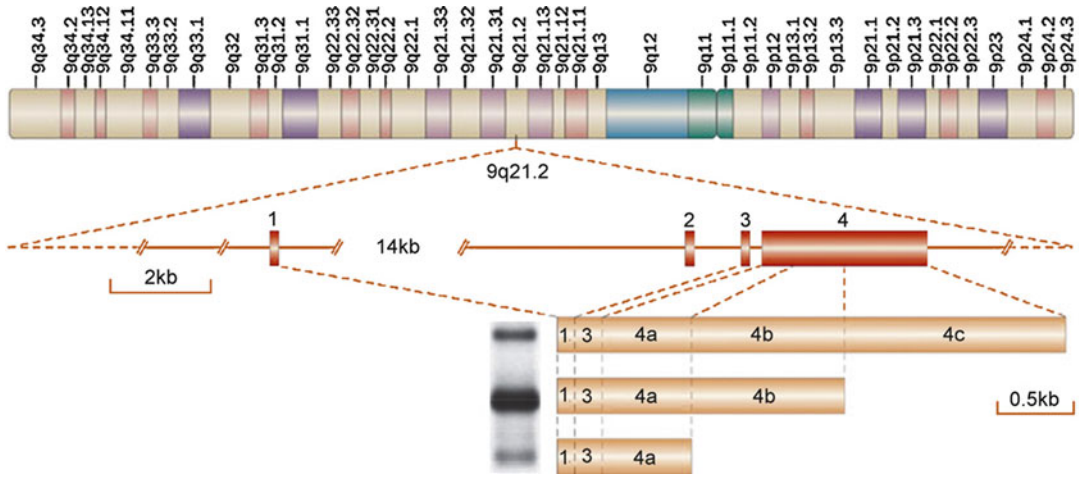

Fig. 17.1 The prostate cancer antigen 3 (PCA3) gene, located at chromosome 9q21-22, consists of four exons, and exon 2 is often skipped by alternative splicing. Three alternative polyadenylations can occur in exon 4 (4a, 4b, and 4c). Most frequently, the transcript contains exons 1, 3, 4a, and 4b (Reprinted with permission from Nature Publishing Group: Hessels et al. Nature Reviews Urology, copyright 2009 [62])

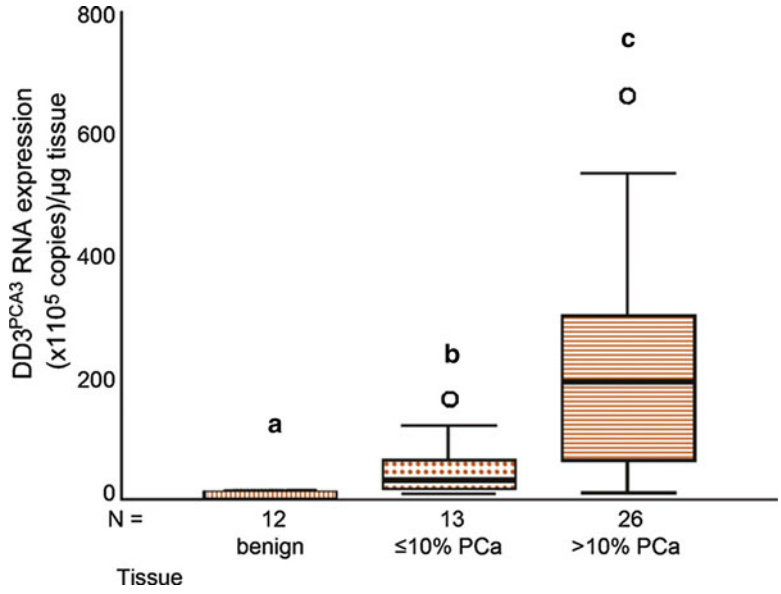

Fig. 17.2 Prostate cancer antigen 3 (PCA3) messenger RNA (mRNA) expression in prostatic tissue. *Box plots* representing the expression of PCA3 mRNA comparing (**a**) benign prostatic tissue (median: 2.4 T 105; range: 0.2 T 105–10.1 T 105); (**b**) prostate tumour containing = 10 % prostate cancer (PCa) cells (median: 25.3 T 105; range: 66.0 T 105–166.0 T 105), and (**c**) prostate tumour containing >10 % PCa cells (median: 158.4 T 105; range: 7.0 T 105–994.0 T 105) (Reprinted with permission from Elsevier: Hessels et al. European Urology, copyright 2003 [26])

measure PCA3 mRNA out of prostate cells shed into urine after digital rectal examination (DRE). Hessels et al. were the first to report of PCA3 mRNA measurement in sedimented urine. PSA mRNA was used to normalise for the amount of prostate specific RNA in the molecular test sample. Although PSA expression is constant in normal cells and 1.5 fold lower in PCa cells, the ratio between PCA3 mRNA over PSA mRNA multiplied by 1000, was presented as a new diagnostic tool – the "**PCA3 score**". In 108 patients, undergoing prostate BX for PCa suspicion based on a PSA level >3 ng/ml, test sensitivity of 67 % and specificity of 83 % were achieved using a determined PCA3-PSA cut-off of 200×10^3 [26].

Tinzl et al. validated the second-generation PCA3 test (uPM3™ assay) comparing urinary

PCA3 to PSA in men undergoing initial and repeat BX for an elevated PSA. In this study the informative rate of 79 % was inferior to current third-generation assays. However, PCA3 achieved a sensitivity and specificity of 82 % and 76 % compared to PSA of 87 % and 16 %, respectively [30]. Using the same assay, the diagnostic superiority of PCA3 was confirmed by Fradet et al. in the first multicenter study including 443 men undergoing prostate BX for elevated PSA. PCA3 vs. PSA revealed an area under the curve (AUC) of 81 % vs. 40 % [28].

In 2006, the prototype of a new quantitative, validated PCA3-based urine test using post-DRE whole-urine specimens further processed in a single-tube format, was presented by Groskopf et al. Urine samples were stored at either 4 °C or 30 °C. The PCA3-to-PSA ratio at 4 °C remained within a 20 % range of the initial values after 2 weeks, but at 30 °C a significant degradation of PCA3 reflected its instability at room temperature. Comparing 52 healthy, 52 BX-negative and 16 BX-positive men, again, median PCA3 mRNA to PSA mRNA ratio values showed significant differences (4.5 vs. 27.0 vs. 81.8; $p<0.01$) [9]. The analytic value oft the new assay was further tested in a multicenter study ($n=179$) conducted by Sokoll et al. Once again, they confirmed the need of an attentive DRE, performed with three or eight strokes ($p=0.85$), to provide high informative test rates up to 95.5 % with total (>18 %), intra-assay (>15 %) and inter-assay (<10 %) variations, respectively. PCA3 scores of BX-positive men showed high correlation when two different research sites were compared (97 %; $p<0.0001$) [29]. Overall, the informative rate of 94–100 % [9, 12, 29–33] of the third-generation PCA3 assay (Progensa®, Hologic Gen-Probe Inc., Bedford, MA, USA) was significantly improved compared to the previously reported ones [27].

The CE approved the PCA3 test in 2006 and finally, the U.S. Food and Drug Administration (FDA) followed in February 2012, to "help clinicians in counselling and determine initial and repeat biopsy indications".

17.3 Clinical Applicability of Prostate Cancer Antigen 3

17.3.1 Early Detection of Prostate Cancer: Initial and Repeat Prostate Biopsy

A main limitation of early PCa detection due to elevated PSA levels remains the high proportion of men detected with non-malignant findings at first or subsequent BX [5, 34]. One of the most important clinical rationales of PCA3 application therefore is the reduction of potentially unnecessary BXs.

Marks et al. evaluated the diagnostic ability of PCA3 in 226 men subjected to repeat BX. They demonstrated PCA3's superiority over PSA in predicting positive BX outcome (AUC: 0.68 vs. 0.52; $p=0.008$). Using 35 as PCA3 score cut-off, a sensitivity, specificity and odds ratio of 58 %, 72 % and 3.6, respectively, was obtained. But, compared to earlier studies [25, 26] median PCA3 scores in aggressive PCa (Gleason score (GS)<7 vs. GS>7) were not significantly different [11]. In contrast, de la Taille et al. have shown in the initial BX setting that the PCA3 score was significantly higher in men with GS 7 or greater vs. GS less than 7 [35].

Consequently, prospective U.S. and European multicentre trials were conducted in patients undergoing initial or repeat BX [10, 13] (Fig. 17.3). As a result comparable diagnostic accuracies of U.S. and European men at first and repeat BX were reported (AUC: 0.68 vs. 0.65). Despite some conflicting results, both studies demonstrated that a combination of PCA3 with established BX risk factors such as age, PSA, DRE, prostate volume and Percent free PSA (%fPSA) improved the predictive accuracy in multivariable regression models. Ploussard et al. performed a subgroup analyses of the European multicenter study and confirmed the superiority of PCA3 over %fPSA in univariable analysis as a predictor of repeat BX outcome (AUC: 0.69 vs. 0.57) [36].

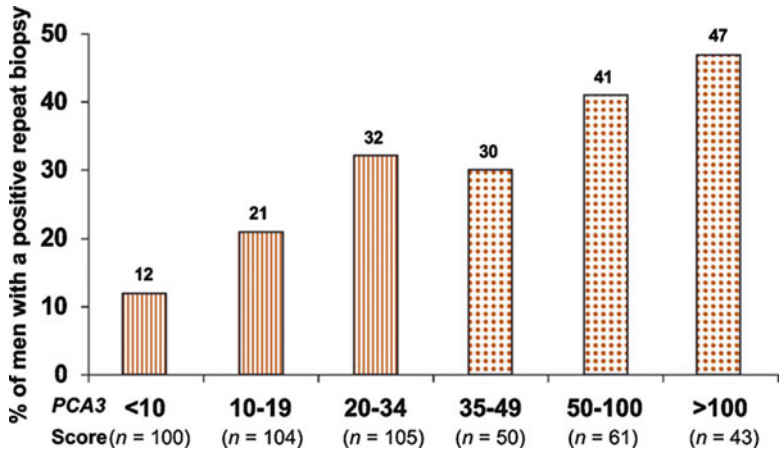

Fig. 17.3 The correlation of prostate cancer antigen 3 (PCA3) to repeat biopsy outcome. *Bars* represent the probability of a positive repeat biopsy expressed in percentages according to different PCA3 score ranges (Reprinted with permission from Elsevier: Haese et al. European Urology, copyright 2008 [10])

The incorporation of PCA3 in the Prostate Cancer Prevention Trial risk calculator (PCPT-RC) improved the diagnostic accuracy compared with the established BX risk factors (AUC: 0.65 vs. 0.70) [37]. In a large mixed BX patient cohort from Europe and Northern America ($n=809$), Chun et al. following Kattan criteria [38, 39] demonstrated that PCA3 independently predicted PCa, and its addition to established risk factors (age, PSA, DRE, prostate volume, BX history) significantly improved predictive AUC of the base model between 2 % and 5 % [14] (Fig. 17.4). The PCA3 based BX nomogram was further externally validated, showing a comparable gain in predictive accuracy [40]. In the specific initial BX setting, an initial BX-specific nomogram has been developed, showing similar findings (AUC 78.1–80.7 %). However, unlike the mixed BX nomogram, the latter initial-BX specific nomogram has also been tested in terms of its ability to avoid unnecessary biopsies without missing high grade PCa. At an exemplary nomogram–derived probability cut-off of 20 %, only 2 % of men with high-grade PCa would be missed, while avoiding up to 55 % of unnecessary prostate BXs [41]. A similar predictive accuracy gain in multivariable analysis has been previously reported by de la Taille et al. where inclusion of PCA3 in multivariable models increased the predictive accuracy by up to 5.5 % [35].

Perdona et al. compared the updated PCPT-RC, including PCA3 and Chun's PCA3-based nomogram. A significantly better discriminative power (AUC: 0.80 vs. 0.72; $p=0.04$) and superior calibration was demonstrated. Decision curve analysis revealed a higher net benefit for Chun's nomogram, resulting in up to 21 % of avoided unnecessary repeat BXs at the expense of missing up to 6.8 % of cancers [42].

Regarding health care expenses and different reimbursement systems in different European countries, at the moment urinary PCA3 measurement is more expensive than PSA measurement. Up-to-date costs for urinary PCA3 testing may be up to 15-fold higher. But, due to PCA3's use to avoid up to 67 % of repeat BXs compared with PSA [10] the avoided BX expenses and further follow-up diagnostic interventions should be considered. Moreover, BX-related anxiety, discomfort and complications may be spared [43].

In conclusion, PCA3 performs as a reliable predictor of PCa at BX, demonstrating superiority over PSA and %fPSA. In combination with established risk factors, PCA3 showed improved accuracy and applicability of new diagnostic tools to assist clinicians in BX decision-making in men who already met established criteria for BX (e.g. elevated PSA, abnormal DRE).

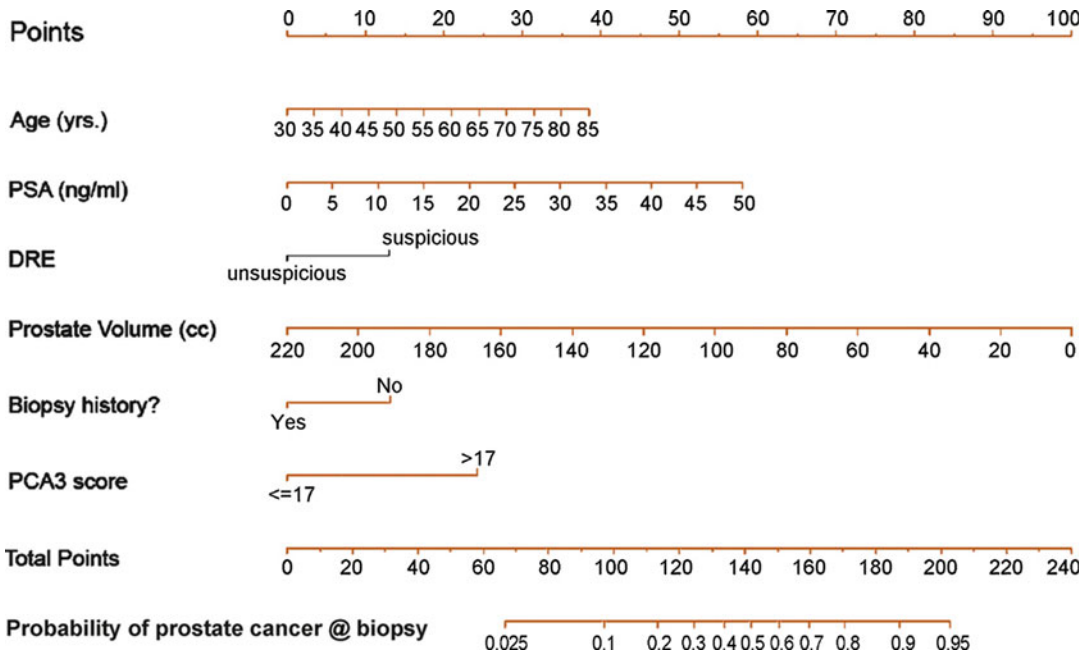

Fig. 17.4 The prostate cancer antigen 3 (PCA3) biopsy nomogram. This recently externally validated nomogram combines established biopsy risk factors such as age, digital rectal examination (DRE), total serum prostate-specific antigen (PSA), prostate volume, and history of previous biopsy together with PCA3 score to predict cancer on prostate initial and repeat biopsy. Instructions for physicians: To obtain nomogram-predicted probability of prostate cancer, locate patient values at each axis. Draw a *vertical line* to the "Point" axis to determine how many points are attributed for each variable value. Sum the points for all variables. Locate the sum on the "Total Points" line to be able to assess the individual probability of cancer on prostate biopsy on the "Probability of prostate cancer at biopsy" line (Reprinted with permission from Elsevier: Chun et al. European Urology, copyright 2009 [14])

17.3.2 Screening and Active Surveillance

PCA3 was assessed as a first-line screening test within the European Randomised Study of Screening for Prostate Cancer (ERSPC) trial. A PCA3 score ≥10 demonstrated a positive predictive value of 17.1 compared with 18.8 for a PSA value ≥3.0 ng/ml. Interestingly, PCA3 versus PSA missed substantially fewer cancers (32 % vs. 65 %) and serious cancers (26 % vs. 58 %). Because this unique study evaluated a PSA-prescreened cohort (third round or more; 33 % had a negative first BX), a consecutive study in unscreened patients, avoiding attribution bias, should be conducted to further assess PCA3 as a potential screening marker [19].

Recently, Tosoian et al. assessed PCA3's ability to rule out clinically significant PCa in men undergoing AS according to the criteria for clinically significant PCa defined by Epstein et al. [44]. A trend towards higher median PCA3 scores in patients with GS upgrading at follow-up BX (72 vs. 50.8; $p=0.08$) was recorded. However, at adjusted multivariable Cox regression analysis, PCA3 did not represent an independent risk factor of BX progression ($p=0.15$) [12]. Considering the limitations that the number of events was small ($n=38$) and that PCA3 was assessed only once at the time of first diagnosis but not repeated during the follow-up biopsies, so far no evidence for the usefulness of PCA3 in AS programs has been presented. Since PCA3 does not appear to represent a useful marker to monitor PCa aggressiveness at biopsies [11, 13] its role in risk assessment during AS needs to be tested in larger studies with repeated PCA3 score measures.

17.3.3 Prediction of Pathological Tumour Volume, Stage and Grade

Du to the fact that PCA3 is highly overexpressed in PCa tissue and improves the prediction of BX outcome, several studies have focused on its potential ability to predict pathological PCa stage and aggressiveness: Bostwick et al. at first reported on 24 patients undergoing radical prostatectomy (RP) for PCa based on a suspicious uPM3™ test. The assessed RP specimens demonstrated no difference in cancer volume, location, stage, and GS compared with RP specimens of men diagnosed with PCa based on PSA or suspicious DRE findings [45].

Using the Progensa® PCA3 assay, Nakanishi et al. analysing 83 RP samples, reported that the urinary PCA3 score significantly correlated with tumour volume (TV), GS, and independently predicted small-volume diseases (TV < 0.5 ml) (AUC: 0.76). Using 25 as a PCA3 score cut-off to predict small-volume tumours in combination with low grade (GS < 7) resulted in a sensitivity and specificity of 70 % and 73 %, respectively. However, it is important to note that the number of events was limited ($n = 10$) [15]. Similarly, Whitman et al. confirmed PCA3's correlation to TV and identified it as an independent predictor ($p < 0.01$) of extracapsular extension (ECE) resulting in a multivariable AUC of 0.90 when combined with PSA and BX GS [16]. In contrast to Nakanishi, Whitman et al. could not find a significant association of PCA3 with pathologic GS [16]. Hessels et al. and van Gils et al. demonstrated neither a significant correlation of PCA3 to pathologic grading nor to TV and pathologic stage in a cohort combining 132 patients [17, 46].

At present, the largest ($n = 305$) published series on urinary PCA3's correlation to clinicopathologic features demonstrated that the multivariable AUC of low-volume disease (+2.4 % to +5.5 %) and insignificant PCa models (+3 % to +3.9 %) improved when PCA3 was added to standard clinical risk factors (Fig. 17.5). On the other side, there was no significant correlation between PCA3 and adverse features such as ECE and seminal vesicle invasion, and its significance on aggressive PCa (RP GS ≥ 7) was reported to be limited [18]. Similar results were reported on

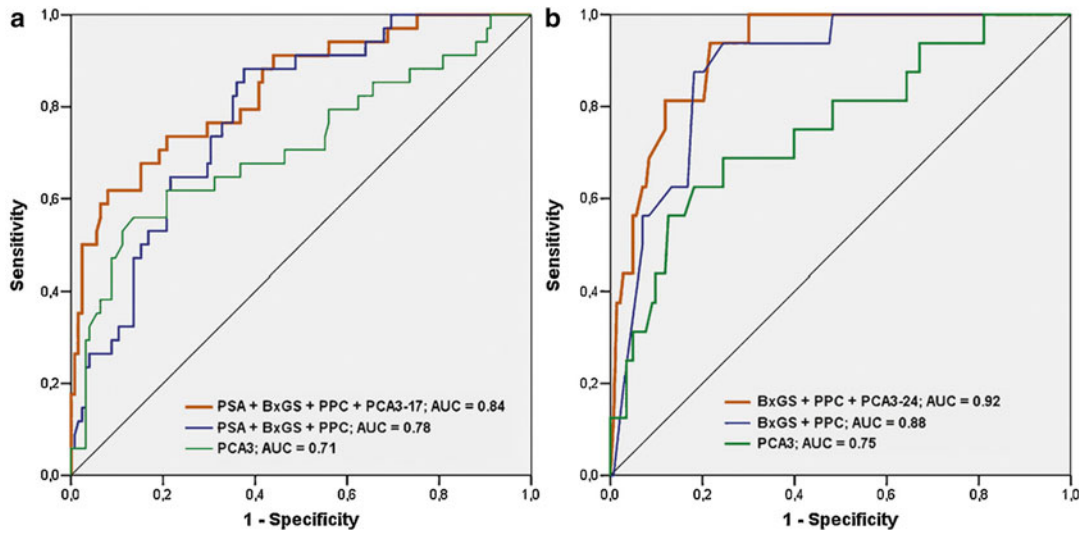

Fig. 17.5 Prediction of small-volume and insignificant prostate cancer with preoperative prostate cancer antigen 3 (PCA3). Receiver operating characteristic curve analyses and area under the curve (AUC) for predicting (**a**) tumour volume <0.5 ml and (**b**) pathologically confirmed insignificant prostate cancer. B × GS = biopsy Gleason score; PPC = percentage of positive cores; PSA = prostate-specific antigen (Reprinted by permission from Elsevier: Auprich et al. European Urology, copyright 2011 [18])

106 consecutive men undergoing RP due to clinically low-risk disease (PSA < 10 ng/ml, T1c–T2a, and biopsy GS < 7). Low urinary PCA3 scores and favourable BX criteria (<33 % or 3-mm tumour; <3 positive cores) independently predicted small TV (<0.5 ml) and insignificant PCa. Again, the urinary PCA3 score, combined with established risk factors in multivariable logistic regression models, was not significantly associated with high-grade and locally advanced disease [47].

Higher PCA3 scores are supposed to be associated with more aggressive cancer, which is based on the hypothesis that with increasing dedifferentiation, PCa cells become more invasive and could therefore more easily be shed into the ductal system of the prostatic gland after DRE or that larger tumours simply have more surface area left to shed PCA3 [46, 47]. Most studies, especially in RP cohorts, failed to confirm this hypothesis [16–18, 46, 47]. But, following GS system [48], some authors suggest that tumours with pattern 4 and 5 increasingly lose their glandular differentiation and lumina, disabling cells to be shed into urine after DRE in correlation with their TV. Therefore, potentially higher PCA3 mRNA tissue levels, resulting from larger tumour masses, might not be adequately measured by the urinary test [18].

In conclusion, evaluations on the potential prognostic role of PCA3, which are currently based on a relatively small number of patients, revealed that it independently predicts small-volume and insignificant PCa. However, PCA3 is not significantly associated with locally advanced disease and has limited value in the prediction of aggressive tumours.

17.3.4 Prostate Cancer Antigen 3 Score Alterations Over Time and Consequence for Bioptic or Medical Intervention

Within the placebo arm of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial, urinary PCA3, PSA and %fPSA were available at the year 2 and year 4 follow-up BX in 1072 men (age: 50–75 years; PSA: 2.5–10 ng/ml; one previous negative 6- to 12-core BX). On univariable analyses for the prediction of year 4 BX outcome based on year 2 biomarker values, PCA3 score was exclusively found as a significant predictor for a positive follow-up BX at year 4. Interestingly, PCA3 scores in BX-positive men only slightly increased (+15.7 %) within the study period [33].

Urinary PCA3 scores before and 2 h after BX, showed no significant difference of measured PCA3 scores, neither in all men (18 %; $p > 0.05$) nor in PCa-positive men (1.5 %; $p > 0.05$) [49]. Sokoll et al. [29] suggest a certain robustness of PCA3 towards interventional effects on the prostatic tissue. In this context, the influence of Dutasteride (5a-reductase inhibitor [5-ARI]) on prostatic markers was assessed by van Gils et al.: In 16 men with BPH and 9 men with clinically localised PCa (all treated with 5-ARI), PSA, testosterone, dihydrotestosterone (DHT), and urinary PCA3 were measured at baseline and after 1, 2 and 3 months. As expected, Dutasteride reduced DHT (>90 %), halved PSA levels, decreased prostate volume (10–16 %), and increased testosterone (20–30 %). In contrast, 5-ARI treatment had a widely variable effect on PCA3 scores, which increased (75–284 %) and decreased (14–77 %) over time, irrespective of whether patients with or without PCa were observed [50]. This needs to be taken into account when counselling patients on Dutasteride who are designated for a PCA3 test.

17.4 New Perspectives

17.4.1 Combination of Prostate Cancer Antigen 3 with New Biomarkers

Since PCA3 is highly PCa specific and a clinically useful marker to predict BX outcome, its combined use with other new tumour markers may further improve its diagnostic accuracy. Therefore, transcripts of a fusion between the transmembrane-serine protease gene (TMPRSS2) and the v-ets erythroblastosis virus E26 oncogene

(ERG) were evaluated in combination with PCA3 in the post-DRE urine of 108 patients undergoing BX. In this study TMPRSS2-ERG fusion transcripts were only found in 59 % of the primary PCa tissue specimens and the included patients did not represent a typical BX cohort because PCa detection rate was quite high with 72 % due to PSA levels ranging from 1.1 to 1619 ng/ml. Urine sediments of men diagnosed with PCa were positive for TMPRSS2-ERG fusion transcripts and PCA3 (cut-off: 48) in 37 % and 62 %, respectively. Combining both markers improved the sensitivity to 73 %, yet a considerable decreased specificity of 63 %, compared with 93 % of TMPRSS2-ERG fusion alone [51].

Laxman et al. further evaluated Golgi membrane protein 1 (GOLM1), serine peptidase inhibitor Kazal type 1 (SPINK1), PCA3 and TMPRSS2-ERG fusion in sedimented urine of men before BX ($n=216$) or RP ($n=60$). A multivariable regression model for the detection of PCa including these four biomarkers improved the diagnostic AUC from 0.66 (for PCA3 alone) to 0.76, respectively [52]. When a-methylacyl-coenzyme racemase (AMACR) and PCA3 from post-DRE urine was assessed in patients undergoing BX due to suspicion of PCa, both markers demonstrated an improved AUC over PSA (0.65 vs. 0.67 vs. 0.59). Using AMACR (cut-off: 10.7) and PCA3 (cut-off: 19.9) within a combined model resulted in a high sensitivity and specificity of 81 % and 84 % vs. 70 % and 71 % vs. 72 % and 59 % for AMACR vs. PCA3 alone, respectively [53]. Rigau et al. using PCA3 together with prostate-specific demonstrated comparable findings G-protein coupled receptor in urine sediments after prostatic massage from 215 patients presented for BX. An increased specificity of 44 % at an assumed sensitivity of 90 % was reported for the combined test compared with each biomarker used as a stand-alone test (25 % vs. 24 %) [54]. Despite the fact that the reported studies used PCA3 cut-off values (19.9, 48) different from the more established cut-off value of 35 reported in previous studies [10, 11, 13, 14, 36, 40], a substantial improvement in the prediction of BX outcome was demonstrated by combining PCA3 and new biomarkers in a limited number of patients. If these promising results could be confirmed by further studies, combinations of new biomarkers including PCA3 may potentially offer an interesting new perspective on the early detection and staging of PCa. However, because to date most of the markers combined with PCA3 are still in their experimental phase, it remains to be assessed which marker panel has the greatest potential to improve predictive ability compared to established markers.

17.4.2 Detection of Prostate Cancer Antigen 3 in Circulating Tumour Cells

In PCa patients, the presence of circulating tumour cells (CTCs) appears to be correlated with a poor prognosis [55]. For this reason detection of specific biomarkers found in prostatic CTCs could potentially indicate an advanced and aggressive stage of disease. In 2008, Väänänen et al. described a quantitative RT-PCR assay for the detection of PCA3 mRNA in peripheral blood and evaluated 67 patients with locally advanced ($n=23$) and metastatic disease ($n=9$), respectively. Interestingly, only two patients were found positive for PCA3 mRNA in peripheral blood samples [56]. In contrast, Marangoni et al. detected PCA3 mRNA expression in 25 (62.5 %) of 40 patients with PCa compared with 15 (37.5 %) of 40 BPH patients by evaluating preoperative peripheral blood samples [57]. Patients presenting with progressive castrate-resistant PCa demonstrated significantly overexpressed levels of PCA3 in CTCs from peripheral blood [58]. Similar findings have been reported by Jost et al. using an immuno-magnetic CTC enrichment method to assess peripheral blood from 67 PCa patients. Although none of the androgen-dependent patients has been tested positive for PCA3, 5 (31 %) of 16 androgen-independent patients were found positive for CTC-PCA3 [59]. In summary, detection of PCA3 mRNA expression in CTCs from peripheral blood samples has been proved to be feasible, although its value in identifying patients with poor prognosis is still

unclear due to limited data. Therefore, further studies are needed.

17.4.3 Prostate Cancer Antigen 3 as a Novel Gene Therapy Target

Van der Poel et al. have demonstrated the high PCa specificity of PCA3 and highlighted its potential use as a precursor to suicide gene therapy by using a specific diphtheria toxin model [60]. A combination of PCA3's promoter region driving the expression of a suicide gene could be used to process novel PCa therapies. In theory, this combined therapeutic construct would bind, interact and finally induce cell death in PCa tissue, and non-malignant and non-prostatic cells would not be affected by this highly specific therapeutic cascade. Based on this concept, Fan et al. developed an oncolytic adenovirus (Ad.DD3-E1A-IL-24), in which replication is driven by the $PCA3^{DD3}$ promoter, carrying the therapeutic gene interleukin (IL)-24. Its in vitro and in vivo effects have been investigated in DU-145 cell lines and in DU-145 xenograft tumours in nude mice. In five of six treated mice, tumours have been completely eliminated within 50 days. Most remarkably, all mice have survived until the end of observation [61]. Despite non- negligible discrepancies regarding the therapeutic effect of Ad.DD3-E1A-IL-24 in vitro and in vivo, this study has demonstrated "Gene-ViroTherapy's" excellent antitumoural efficacy in an initial small single tumour model study in mice. Therefore further investigations on PCA3's potential role in PCa gene therapy should be intensively promoted in the future.

17.5 Conclusions

PCA3 has shown its potential to assist clinicians in patient counselling and BX indication confirmation in men at risk for PCa based on elevated serum tPSA levels and/or suspicious DRE. Ideally, PCA3 would be used in combination with other established PCa risk factors to combine each marker's strengths to focus on detection of significant disease that is more likely to be cured if detected early. The value of PCA3 for prediction of PCa significance remains controversial. It does not appear to be associated with advanced disease and PCa aggressiveness in men undergoing RP, and its use as a follow-up marker for AS patients does not seem to be given. The main current indication of the PCA3 urine test may be to determine whether a man needs a (repeat) BX after an initially negative BX outcome, albeit its cost-effectiveness remains to be shown. Finally, the implementation of the PCA3 promoter in developing new highly PCa-specific gene therapies represents a promising perspective in the near future.

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