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## ORIGINAL ARTICLE

# Age-Adapted Prostate Cancer Gene 3 Score Interpretation - Suggestions for Clinical Use

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**SUMMARY**

***Background:*** **The prostate cancer antigen 3 (PCA3) gene urine assay is established for biopsy decision in case of prostate cancer (PC) suspicion. Recent findings pointed to an age dependence of PCA3, with putative impact on test interpretation. However, to date no experience has been reported with regard to the extent age might modify the score in certain age ranges. Therefore, the aim of the present study was to re-evaluate the age dependency and, moreover, give suggestions for interpretation of the PCA3 score in dependence of patient's age in daily routine.**
***Methods:*** **The study comprised 684 patients before prostate biopsy or prostatectomy. Post-massage voided urine samples were assessed by PCA3 measurement. PCA3 scores were correlated to patient's age. The collective was divided into four subcollectives by quartiles of age distribution. For every subcollective the cutoff value at specificity of ≥ 60 was determined. Results were classified by age-class specific cutoff values and test qualities were compared at different cutoffs.**
***Results:*** **In the collective, 59.1% of patients had a positive biopsy. PCA3 correlated to patient's age in univariate and multivariate analysis ($p < 0.001$ each). The division into age subcollectives revealed groups < 60, 60 - 65, 66 - 69 and > 69 years. Median PCA3 values of patients without/with PC were 17/32, 27/42, 34/55 and 52/68 in the four age classes. Cutoff values for which specificity was determined with ≥ 60 were 23, 39, 42, and 65. Constant cutoff values showed lower sensitivities in younger and lower specificities in older patients. Only the age adjusted values revealed an improved performance with PPV 68.7, accuracy 59.5 and sensitivity 57.7 at specificity of 62.1% in the whole cohort.**
***Conclusions:*** **The study confirms that the PCA3 score increases with age. The recommended cutoff score of 35 is suitable especially for patients aged in their sixties. Lower reference values between 20 and 30 have to be taken into account in patients aged < 60 years and higher values around 40 to 50 may point to suspicion for PC in patients > 69 years. These results may further improve the diagnostic performance of the PCA3 test and keep the PCA3 test as a significant test in PC diagnostics along with new upcoming urine markers.**
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**KEY WORDS**

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## INTRODUCTION

Prostate cancer (PC) remains one of the most prevalent cancers in men. In PC diagnostics one of the main problems consists in artificially elevated levels of serum total prostate specific antigen (tPSA) due to non-malig-

nant causes. To avoid unnecessary prostate biopsies, the prostate cancer gene 3 (PCA3) urine assay [1] was introduced as the first urine based molecular test to close this diagnostic gap for PC [2]. The test has shown to be a valid [1] discriminative marker for biopsy decision thereby exceeding tPSA (alone) [3]. The test is based on the transcription-mediated amplification method [4] and reveals a continuous variable resulting from a quotient of PCA3/PSA mRNAs. For biopsy decision, a cutoff value of 35 has been recommended. However, there is ongoing discussion on the diagnostic accuracy of this reference value [5,6].

Like every diagnostic system, the PCA3 assay may be subjected to influencing factors that may alter the result. In earlier studies, the test has been described as not influenced by prostate volume [7] or inflammation [8]. Interestingly, Klatte et al. found an age dependence of the PCA3 score independent of prostate volume in a collective of 205 patients [9]. PCA3 has been shown to increase with patient's age, independent of the presence of PC. They constructed multivariate models and showed superiority of age-related adjustments revealed from linear regression analyses of biopsy negative patients. However, as the authors point out, it was not the aim of their study to propose new cutoff values for each age or age decade.

Therefore, currently there are no precise suggestions for age adapted reference values of PCA3 available. The present study aims to propose distinct age dependent values for considering them in practical use and thereby investigate which adjustments have to be conducted in certain age cohorts.

## MATERIALS AND METHODS

### Patients collective

The study comprised a collective of 684 consecutive patients with a PCA3 assessment with suspicion of PC prior to prostate biopsy due to elevated tPSA and/or positive digital rectal examination and patients with proven PC before prostatectomy at the University Hospital of Tübingen, Department of Urology.

In the biopsy collective, patients whose previous biopsy was closer than six months and patients with known PC or other urogenital melanoma were excluded. Patients with less than 10 core biopsies and patients not able to undergo prostate massage or provide voided urine were excluded. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the local ethics committee (No. 538/2018BO2).

### Assessment of patients' clinical and pathological data

The following patients' data were collected: patient's age, prostate volume determined by transrectal ultrasound (TRUS), tPSA value, and biopsy results including postoperative pT stage and prostatectomy Gleason score.

### Retrieval of urine samples and processing

Patients underwent a digital rectal examination of the prostate according to the protocol of the PCA3 manufacturer [10]. Both lobes of the prostate were streaked out three times with medium pressure on both sides, from the base to the apex and from the lateral sides towards the mid-line. A first stream urine sample of 2.5 mL was collected and poured into a transport tube containing the stabilizer fluid (Progensa™ Urine Specimen Kit, HOLOGIC®, Marlborough, MA, USA). The samples were stored according to the manufacturers' protocol and final analysis was carried out within five days after sample retrieval.

### Determination of PCA3

The samples were analyzed in the laboratory of the Department of Urology under application of the commercially available Progensa™ PCA3 analysis system (HOLOGIC® PCA3 assay, HOLOGIC®) according to the manufacturer's recommendations.

### Statistical data analysis

Patients with PCA3 scores > 200 were excluded from the study. Statistical analysis was conducted using commercially available software JMP (14.2., SAS Inc., Cary, NC, USA). Statistical differences were considered significant with $p < 0.05$. Age and PCA3 score were correlated by linear regression analyses and nonparametric Spearman's ρ test. To exclude the influence of known modulation factor malignancy on the PCA3 score and known elevation of prostate volume with age, multivariate logistic regression models were created including these two parameters.

The study cohort was further divided into four subcollectives by the quartiles of patients' age distribution. The PCA3 scores were compared between age classes by nonparametric Wilcoxon-Kruskall-Wallis test and Cochran-Armitage test for trend at a cutoff score of 35. Receiver-Operating-Characteristic (ROC) curves were generated for the whole cohort and the four subcollectives.

The area under the curve (AUC) was calculated and sensitivities, specificities, negative predictive values (NPVs), positive predictive values (PPVs), and accuracies were calculated for every step of the ROC table. The cutoff value at which the specificity counted ≥ 60 was determined for each age class. Test qualities were calculated for the whole cohort and the subcollectives at a cutoff score of 35, at a cutoff of 20, at the best cohort cutoff, and at a specificity ≥ 60. Additionally, test qualities were calculated for the whole cohort using the age class specific cutoff value that revealed a specificity ≥ 60 for every patient.

**Table 1. Patients' characteristics of the total cohort and age-dependent subcollectives.**

| n | 684 | 156 | 183 | 152 | 193 |
|---|---|---|---|---|---|
| Age, median (range), years | 66 (40 - 82) | 56 (40 - 59) | 63 (60 - 65) | 68 (66 - 69) | 72 (70 - 82) |
| Prostate volume, median (range) mL | 49 (3 - 255) | 35 (10 - 132) | 40 (6 - 153) | 40 (10 - 110) | 42 (3 - 255) |
| tPSA, median (range), ng/dL | 6.6 (0.1 - 210.1) | 7.2 (0.4 - 89.6) | 6.7 (0.3 - 31.8) | 6.6 (0.1 - 72.0) | 6.2 (0.5 - 210.0) |
| Prostate cancer, n (%) | 404 (59.1) | 94 (60.3) | 103 (56.3) | 98 (64.5) | 109 (56.5) |
| Prostatectomy pT Stage, pT2/pT3 (%) | 238/79 (75.1/24.9) n = 317 | 56/23 (70.9/29.1) n = 79 | 69/19 (78.4/21.6) n = 88 | 55/17 (76.4/23.6) n = 72 | 58/20 (74.4/25.6) n = 78 |
| Postoperative Gleason score, < 8/≥ 8 (%) | 342/49 (87.8/12.2) n = 401 | 83/11 (88.3/11.7) | 89/13 (87.3/12.7) n = 102 | 92/6 (93.9/6.1) | 88/19 (82.2/17.8) n = 107 |
| PCA3, median (Range) | 42 (1 - 199) | 23.5 (1 - 168) | 38 (2 - 199) | 47.5 (5 - 193) | 61 (1 - 199) |

**ng/dL - nanogram per deciliter, mL - milliliter.**

**Table 2. Results from the multivariate model analysis. Estimated impact of listed variables and result from interaction effect analysis (age - biopsy result).**

| Term | Estimate | STD | t-value | FDR - adjusted p-value |
|---|---|---|---|---|
| Whole model | | | | < 0.0001 |
| Age | 1.91 | 0.21 | 8.90 | < 0.0001 |
| Prostate volume | -0.05 | 0.07 | -0.71 | 0.64 |
| Biopsy result | 7.94 | 1.62 | 4.90 | < 0.0001 |
| Interaction age - biopsy result | 0.01 | 0.21 | 0.01 | 0.995 |

**STD - standard deviation, FDR - false discovery rate.**

## RESULTS

Median PCA3 scores in the cohorts without and with PC were 33 (1 - 199) and 50 (1 - 199, $p < 0.001$), respectively. Characteristics of the total cohort and the age-dependent subcollectives are given in Table 1.

The PCA3 score correlated to age in both groups without and with PC ($p < 0.0001$ each, $r^2 = 0.13$ and 0.09, linear adjustment equations were PCA3 = 1.9 x age - 79 and PCA3 = 1.9 x age - 69), respectively (Figure 1AB). In the multivariate model, age was identified as an independent predictor of PCA3 increase (1.9 PCA3 score unit increase per year). In addition, biopsy result was a strong independent predictor of PCA3 score with an estimated elevation of 7.9 in case of PC ($p < 0.001$ each, FDR-adjusted) with no impact of prostate volume. An interaction between age and biopsy result could not be determined (Table 2).

The division into four subcollectives revealed groups < 60, 60 - 65, 66 - 69 and > 69 years. Figure 2 shows PCA3 levels in the four age classes separated for patients with negative biopsy and with PC ($p < 0.001$ each).

ROC curve analyses were performed for the whole cohort and in each age class. The AUC for the whole cohort was 0.632. Age dependent subgroup analysis showed AUCs of 0.684 (< 60 years), 0.650 (60 - 65), 0.688 (66 - 69) and 0.572 (> 69) with best cutoffs at scores 19, 24, 42, and 37, respectively.

To avoid unnecessary biopsies, the positive predictive value and specificity should be as high as possible. Therefore, we determined cutoff values in the age classes at a specificity reaching at least 60%. Cutoff PCA3 scores for which the specificity was ≥ 60% were 23 (< 60 years), 39 (60 - 65), 42 (66 - 69) and 65 (> 69), respectively. These were taken as the basis for age ad-

**Table 3a - e. Test qualities in different age classes. Application of different cutoff values (3a-d) and the age adjusted cutoff resulting from the respective cutoff values at specificity ≥ 60 for each age class (3e).**

| | | Sensitifity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|
| a | Cutoff 35 | | | | | |
| | Whole cohort | 67.3 | 52.5 | 67.2 | 52.7 | 61.3 |
| | Age < 60 years | 47.9 | 72.6 | 72.6 | 47.9 | 57.7 |
| | 60 - 65 years | 63.1 | 56.3 | 65.0 | 54.2 | 60.1 |
| | 66 - 69 years | 75.5 | 50.0 | 73.3 | 52.9 | 66.4 |
| | > 69 years | 80.7 | 35.7 | 62.0 | 58.8 | 61.1 |
| b | Cutoff 20 | | | | | |
| | Whole cohort | 82.7 | 35.0 | 64.7 | 58.3 | 63.2 |
| | Age < 60 years | 68.1 | 59.7 | 71.9 | 55.2 | 64.7 |
| | 60 - 65 years | 80.6 | 42.5 | 64.3 | 63.0 | 63.9 |
| | 66 - 69 years | 90.1 | 25.9 | 69.0 | 60.9 | 67.8 |
| | > 69 years | 89.9 | 15.5 | 58.0 | 54.2 | 57.5 |
| c | Best cohort cutoff, 48 | | | | | |
| | Whole cohort | 52.5 | 68.9 | 70.9 | 50.1 | 59.2 |
| | Age < 60 years | 36.2 | 90.3 | 85.0 | 48.3 | 57.7 |
| | 60 - 65 years | 46.6 | 76.3 | 71.6 | 52.6 | 59.6 |
| | 66 - 69 years | 59.2 | 66.7 | 76.3 | 47.4 | 61.8 |
| | > 69 years | 66.1 | 47.6 | 62.1 | 51.9 | 58.0 |
| d | Cutoff for specificity ≥ 60 | | | | | |
| | Whole cohort 42 | 58.7 | (60.7) | 68.3 | 50.4 | 59.5 |
| e | Cutoff age-adjusted | | | | | |
| | Whole cohort, variable | 57.7 | 62.1 | 68.7 | 50.4 | 59.5 |
| | Age < 60 years 23 | 61.7 | (61.3) | 70.7 | 51.4 | 61.5 |
| | 60 - 65 years 39 | 57.3 | (60.0) | 64.8 | 52.2 | 58.5 |
| | 66 - 69 years 42 | 68.4 | (63.0) | 77.0 | 52.3 | 66.4 |
| | > 69 years 65 | 53.2 | (61.9) | 64.4 | 50.5 | 57.0 |

**PPV - positive predictive value, NPV - negative predictive value.**

justed values in the present analysis.
Table 3a - e shows test qualities for using cutoff a) 35 and b) 20, c) the best cohort cutoff from ROC analysis 48, d) the cutoff for 'specificity ≥ 60%' (42 in the whole cohort), and e) the age adjusted cutoff for each age class resulting from d) (variable).

## DISCUSSION

Multiple trials have shown the usefulness of PCA3 compared with serum PSA for biopsy decision of PC [11]. The PCA3 score is a continuous variable derived from expression analysis and values can be used for interpretation according to experience and recommended reference values, even with different borders for different clinical scenarios such as e.g., high grade prostatic intraepithelial neoplasia or atypical small acinar proliferation [12]. Moreover, interactions with individual, patient associated variables can alter test results and ultimately lead to misinterpretations as seen in a variety of urine based biomarker analyses [13]. Assumed age dependence could be considered by including this information into the test algorithm, thereby revealing an age-related score. This principle has recently also been introduced in molecular PC diagnosis using multivariate

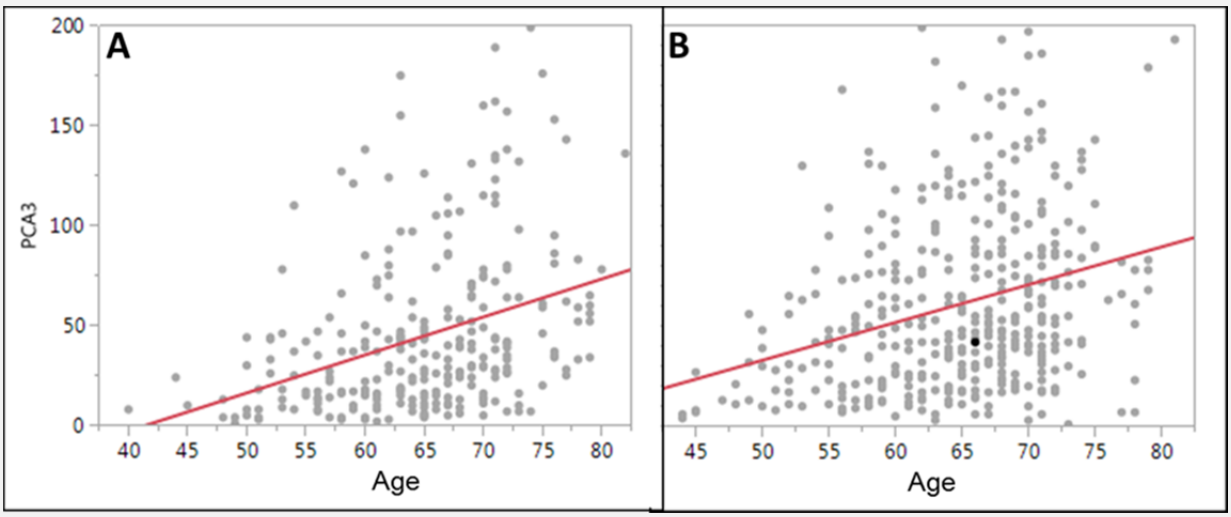


**Figure 1A - B. Linear regression analyses from prostate cancer gene 3 (PCA3) scores versus patients' age.**

**A. Patients with negative biopsy, B. Patients with prostate cancer.**
**Red = fit line, $p < 0.001$ each, $r^2 = 0.13$ and 0.09, linear adjustment equations A: PCA3 = 1.9 x age -79 and B: PCA3 = 1.9 x age -69.**

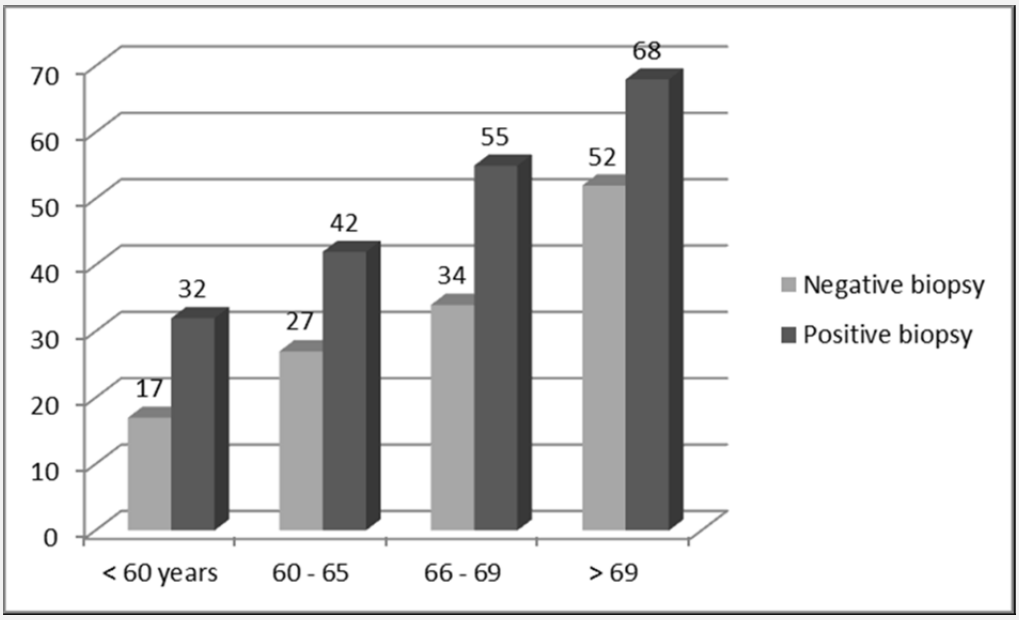


**Figure 2. PCA3 results from samples of different age classes.**

**Medians as columns ($p < 0.001$ each for negative biopsy and positive biopsy).**

clinical and molecular parameters [14]. Since such multivariate models for PCA3 based diagnostics are not available the present work obtains to provide a practical instruction to interpret the results depending on patients' age.

In a substantial collective of 684 patients, the data from the present study confirms previous findings [9] of an age dependence of PCA3 scores, independent from malignancy and prostate volume as this may elevate with age. Interestingly, the inclinations of the fit lines in patients with negative biopsy and PC patients are the same (both 1.9, Figure 1); the lines are only shifted parallel for 10 score points (Figure 1). However, there is an obvious large spread visible in the values. Importantly, the biopsy result was not associated with patients' age; hence, there is no risk of an age-related bias. Median PCA3 scores were elevated with increasing age. Within this, PCA3 scores of patients with PC were shown to be higher by 15 - 20 points (see Figure 2).

Influence of patients' age on PCA3 as a diagnostic marker for PC has been frequently discussed in earlier studies. Martínez-Piñeiro et al. found that in patients with advanced PC the median PCA3 scores ('at baseline') were significantly higher in patients aged ≥ 65 years versus in those aged < 65 [15]. Similarly, Wu et al., using a cutoff score of 25, found patients with false negative results to be generally younger (61.2 versus 66.1 years, $p = 0.08$) [16]. In an editorial comment, E. M. Schaeffer pointed out that predications on PCA3 may only be made on the basis of an appropriate underlying age collective [17]. On the other hand, Haese et al. did not identify differences in PCA3 performance between two age subcollectives: below and ≥ 65 years [2]. However, so far, most studies on PCA3 address age as a variable that impacts nomograms for predicting PC instead of determining the true influence on the score and consequently offering an age-adapted test interpretation strategy.

Given the results from our analysis, there are several observations to note (see Table 3): As expected, the higher the cutoff value, the better the specificity at a concomitant decrease of sensitivity. However, within this, specificity decreased with increasing age and sensitivity decreased with decreasing age. This resulted in problematic values of sensitivity in younger and of specificity in older patients, up to a dramatic 15.5% specificity at cutoff of 20 in patients aged > 69 years. When predefining the specificity > 60%, the PCA3 cutoff scores are increasing with age. When adapting PCA3 score interpretation according to the resulting age-related best cutoffs, relevant decreases of test quality are no longer detectable and PPVs remain in comparable dimensions.

It becomes clear that the good overall test qualities for the cutoff score of 35 arise from a superior performance in certain age classes (60 - 69) whereas in other age ranges the test quality is inferior at the given cutoff score (see Table 3a). Depending on the respective PCA3 cutoff score, test qualities decrease either in younger or in older patients' ages. Only the age adjusted values reveal a good performance of the test within all age classes.

The PCA3 cutoff score, initially proposed as 35 by the manufacturer based on receiver operating characteristic analysis, has been under intense discussion [2]. There were suggestions to lower the cutoff level [18] thereby accepting a reduced specificity and hence the goal to avoid biopsies due to false positive results would fail. As discussed above, it is by nature in diagnostic tests for disease that individual cutoff values result in either limited sensitivity or specificity. The present data shows, that these limitations increase in certain age ranges. Concerning age, the present work clearly points to the use of lower cutoff scores in younger and higher scores in older patients.

The source of this age dependent increase still remains unclear. One may hypothesize on altered expression or stability of PCA3 mRNA alongside aging, altered PSA mRNA expression alongside aging (affecting the score-quotient by diminishing the denominator), altered permeability of the prostatic tissue specific for PCA3 mRNA different to PSA mRNA or unknown urine contents that damage mRNA in the specimen to a different extent alongside aging. As Klatte et al. pointed out, PCA3 scores depend on PSA expression, and PSA is influenced by patients' age [19]. However, this is reported exclusively for the PSA protein and includes the known fact that the prostate volume increases with age and in parallel with the serum tPSA [20]. Furthermore, the prostate is reported to be more 'leaky' for tPSA in older men due to the breakdown of the normal physiological barriers [21]. On the cellular regulation level, Bianchi-Frias et al. found several changes in expression of transcripts in the prostate tissue of older mice: e.g., increased inflammation parameters and, of particular note, decreased collagen genes and also an abundant collagen matrix, all together pointing to molecular changes in the prostate alongside aging including permeability of the tissue [22]. However, even if the elevation of PSA alongside aging would result from an increase in mRNA transcripts, this would result in the opposite condition from that observed: higher PSA mRNA would even lower the PCA3 score in elderly men due to an increase in denominator value of the fraction.

Some limitations of the study should be stated. The classification of the subcollectives led to the ROC based age-adjusted cutoff values used here. A different group division may lead to slightly different results. Furthermore, there is the common bias of including only patients who were led to biopsy. Patients showing elevated PSA levels might be overrepresented. As PSA mRNA is substantial content of the PCA3 score, a potential regulation between the age groups might be of particular limiting note; however, only a marginal regulation in PC is reported. Since this evaluation is derived only from a single center data, the present observation should be re-evaluated in a multicenter scenario.

## CONCLUSION

PCA3 score increases with age. Hence, when interpreting the PCA3 score, urologists can rely on the 35 recommendation for patients aged in the sixties. In patients < 60 years of age, lower PCA 3 scores between 20 and 30 have to be taken into account, while in patients aged > 69 years higher PCA3 levels around 40 to 50 indicate presence of prostate cancer. In the light of other upcoming and approved multivariate molecular tests, these results may contribute to a further improvement of the diagnostic performance of the PCA3 for prostate cancer detection.

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**Declaration of Interest:**
There are no conflicts of interest.

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