

# THE PCA3 ASSAY IMPROVES THE PREDICTION OF INITIAL BIOPSY OUTCOME AND MAY BE INDICATIVE OF PROSTATE CANCER AGGRESSIVENESS

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## Introduction

- In early diagnosis of prostate cancer (PCa), many men with a suspicious digital rectal examination (DRE) and/or chronically elevated serum prostate specific antigen (PSA) level have a negative prostate biopsy (Bx)
- Widespread PSA testing and lowering of the PSA threshold has increased the number of (negative) Bx and resulted in increased diagnosis of clinically insignificant PCa
- Bx is not only costly but can also induce anxiety, discomfort, pain and (sometimes severe) complications<sup>1,2</sup>
- Additional tests are needed to help guide Bx decisions and reduce the number of unnecessary Bx
- There is also need for additional tests to distinguish clinically significant cancers, which need curative therapy, from indolent cancers, which can be actively monitored, and as such reduce overtreatment
- The PROGENSA<sup>®</sup> PCA3 (Prostate CAncer gene 3) Assay is a gene-based, highly PCa-specific test that detects the presence of PCa messenger RNA (mRNA) in urine
- Recent research has shown its clinical utility in men with  $\geq 1$  negative prior Bx in guiding repeat biopsy decisions, as well as its correlation to PCa aggressiveness<sup>3,7</sup>

## Objective

- To evaluate the clinical utility of the PCA3 Assay in guiding initial Bx decisions in men with a PSA 2.5-10.0 ng/mL

## Materials and methods

- A prospective, multi-national, multi-centre study was performed in men with a serum total PSA  $\geq 2.5$ -10.0 ng/mL scheduled for initial Bx
- Post-DRE (3 strokes per lobe), 20-30 mL of first catch urine was collected, 2.5 mL was transferred into a transport tube (Figure 1) and shipped at room temperature to a certified laboratory for determining PSA and PCA3 mRNA concentrations using the PROGENSA<sup>®</sup> PCA3 Assay<sup>®</sup>
- The PCA3 Score was calculated as: [mRNA PCA3] / [mRNA PSA] x 1000
- The PCA3 Score was compared to the Bx outcome ( $\geq 8$  cores) and other clinical findings
- The diagnostic accuracy of the PCA3 Score was compared with that of serum total PSA, PSA density (PSAD) and % free PSA (not performed in all centres)

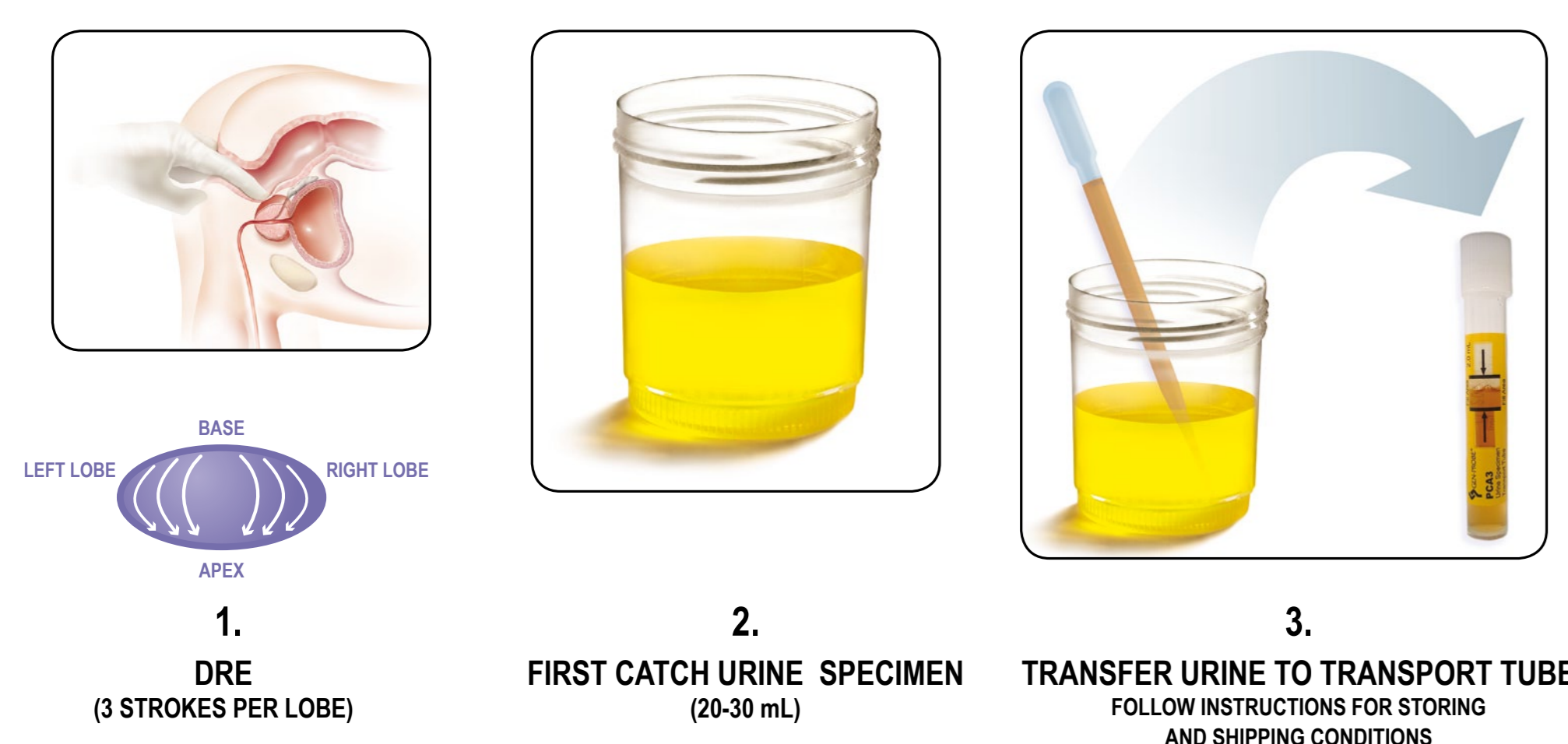


FIGURE 1: THE PCA3 URINE SPECIMEN COLLECTION PROCEDURE

## Results

### Informative rate

- The informative rate was 99% demonstrating robustness of sample collection

### Study population

- 516 men with a conclusive Bx result and PCA3 Score were included in the analysis
- 207 (40%) had a positive Bx
- Baseline characteristics are presented in Table 1
  - The majority of men (85%) had a serum PSA 4-10 ng/mL
  - Of men with a positive Bx
    - 86% had clinical stage T1c, 12% stage T2
    - 94% had a Bx Gleason score of 6-7
    - 59% had  $\leq 33\%$  of total number of cores positive for cancer

TABLE 1: BASELINE CHARACTERISTICS

| Characteristic                                     |                         | Men with Bx- (N=309)    | Men with Bx+ (N=207)    | Pvalue* |
|--|-------------------------|-------------------------|-------------------------|---------|
| Age (years) <sup>1</sup>                           | Mean $\pm$ SD<br>Median | 61.8 $\pm$ 7.2<br>62    | 64.8 $\pm$ 7.8<br>66    | <0.0001 |
| Prostate volume (cc) <sup>2</sup>                  | Mean $\pm$ SD<br>Median | 53.1 $\pm$ 21.9<br>48   | 41.9 $\pm$ 17.2<br>40   | <0.0001 |
| Serum total PSA (ng/mL) <sup>3</sup>               | Mean $\pm$ SD<br>Median | 5.6 $\pm$ 2.0<br>5.2    | 6.2 $\pm$ 2.1<br>5.8    | 0.0029  |
| PSAD (ng/mL/cc) <sup>4</sup>                       | Mean $\pm$ SD<br>Median | 0.12 $\pm$ 0.06<br>0.11 | 0.17 $\pm$ 0.09<br>0.15 | <0.0001 |
| % free PSA (%) <sup>5</sup>                        | Mean $\pm$ SD<br>Median | 18.4 $\pm$ 8.1<br>17.8  | 16.4 $\pm$ 10.5<br>14.0 | 0.0019  |
| Number (%) of men with suspicious DRE <sup>6</sup> |                         | 38 (13%)                | 60 (29%)                | <0.0001 |
| PCA3 Score   | Mean $\pm$ SD<br>Median | 31.0 $\pm$ 46.9<br>18   | 69.6 $\pm$ 73.9<br>50   | <0.0001 |

Bx+: positive Bx; Bx-: negative Bx; SD: standard deviation; \*: Bx+ vs. Bx-; 1: N=308/207; 2: N=275/183; 3: N=307/206; 4: N=273/182; 5: N=165/126; 6: N=304/204

### Performance characteristics

- The area under the curve of the receiver operating characteristics (AUC ROC) of the PCA3 Score was compared with that of serum total PSA, PSAD and % free PSA (Figure 2; Table 2)
- The diagnostic accuracy of the PCA3 Score was statistically significantly better than that of serum total PSA, PSAD and % free PSA
- The greatest diagnostic accuracy of the PCA3 Score was obtained at a cut-off of 35: specificity 76% and sensitivity 64% (Table 3)
- At a sensitivity of 80%, the PCA3 Score specificity of 58% was higher than the 44% for PSAD and 27% for serum total PSA and % free PSA

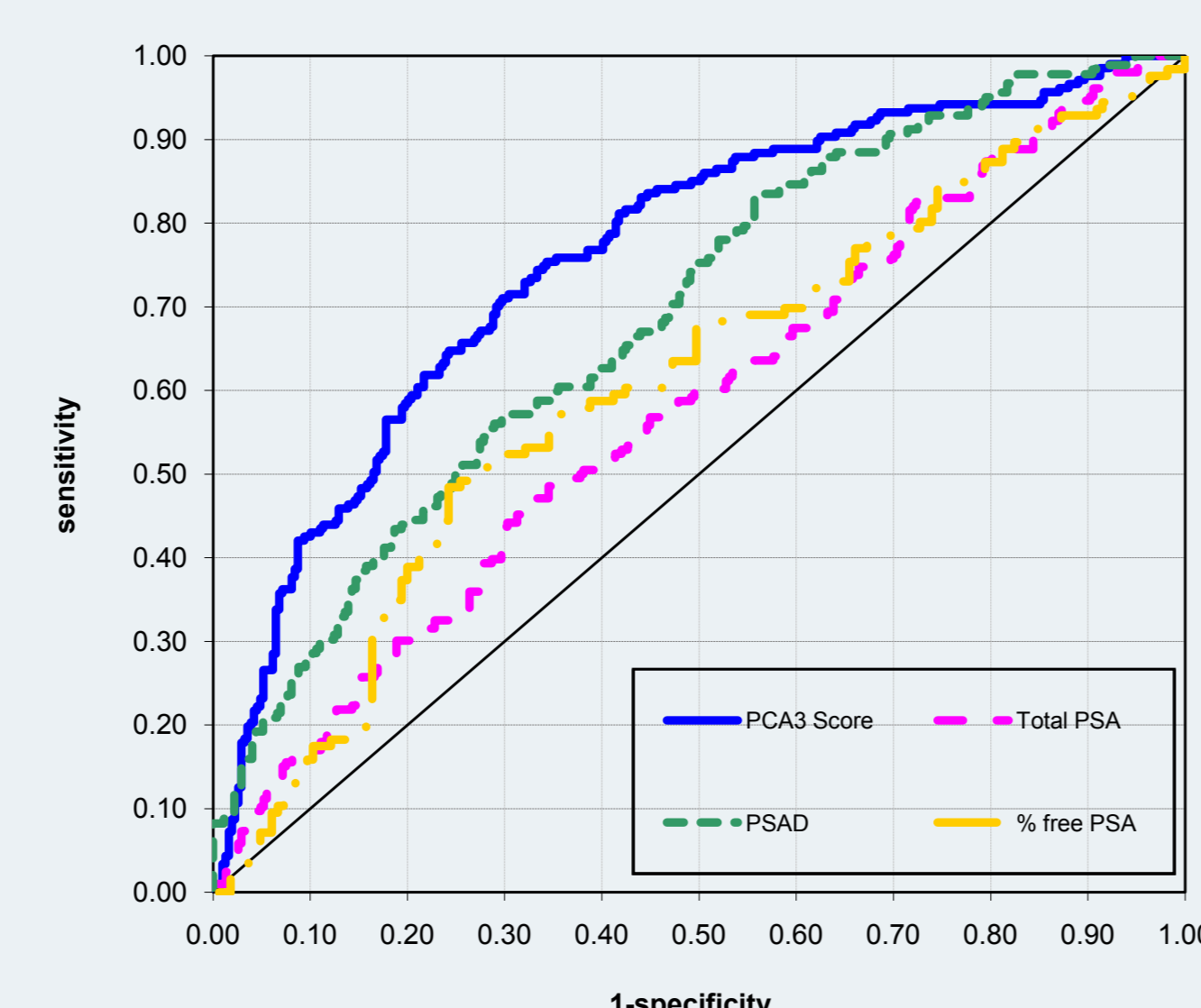


FIGURE 2: THE DIAGNOSTIC ACCURACY OF THE PCA3 ASSAY, SERUM TOTAL PSA, PSAD AND % FREE PSA

TABLE 2: PCA3 HAS A STATISTICALLY SIGNIFICANTLY HIGHER ROC AUC THAN SERUM TOTAL PSA, PSAD AND % FREE PSA

|                 | N   | AUC   | Pvalue* |
|-----------------|-----|-------|---------|
| PCA3 Score      | 516 | 0.761 |         |
| Serum total PSA | 513 | 0.577 | <0.0001 |
| PSAD            | 455 | 0.689 | 0.0232  |
| % free PSA      | 291 | 0.606 | <0.0001 |

\* vs. PCA3

TABLE 3: SENSITIVITY AND SPECIFICITY OF THE PCA3 SCORE, SERUM TOTAL PSA, PSAD AND % FREE PSA

|                                  | Sensitivity | Specificity |
|----------------------------------|-------------|-------------|
| PCA3 Score; cut-off 20           | 84%         | 55%         |
| PCA3 Score; cut-off 35           | 64%         | 76%         |
| PCA3 Score; cut-off 50           | 50%         | 83%         |
| Serum total PSA; cut-off 4 ng/mL | 91%         | 16%         |
| PSAD; cut-off 0.15 ng/mL/cc      | 50%         | 75%         |
| % free PSA; cut-off 25%          | 90%         | 18%         |

\* not performed in all centres (N=291 out of 516)

### Probability of a positive Bx

- Men with a PCA3 Score  $\geq 35$  had an almost 3-fold increased probability of having a positive Bx than those with a PCA3 Score < 35 ( $P < 0.0001$ ; Figure 3)
- The mean PCA3 Score was statistically significantly higher in men with a positive Bx than in men with a negative Bx ( $P < 0.0001$ ; Figure 4)

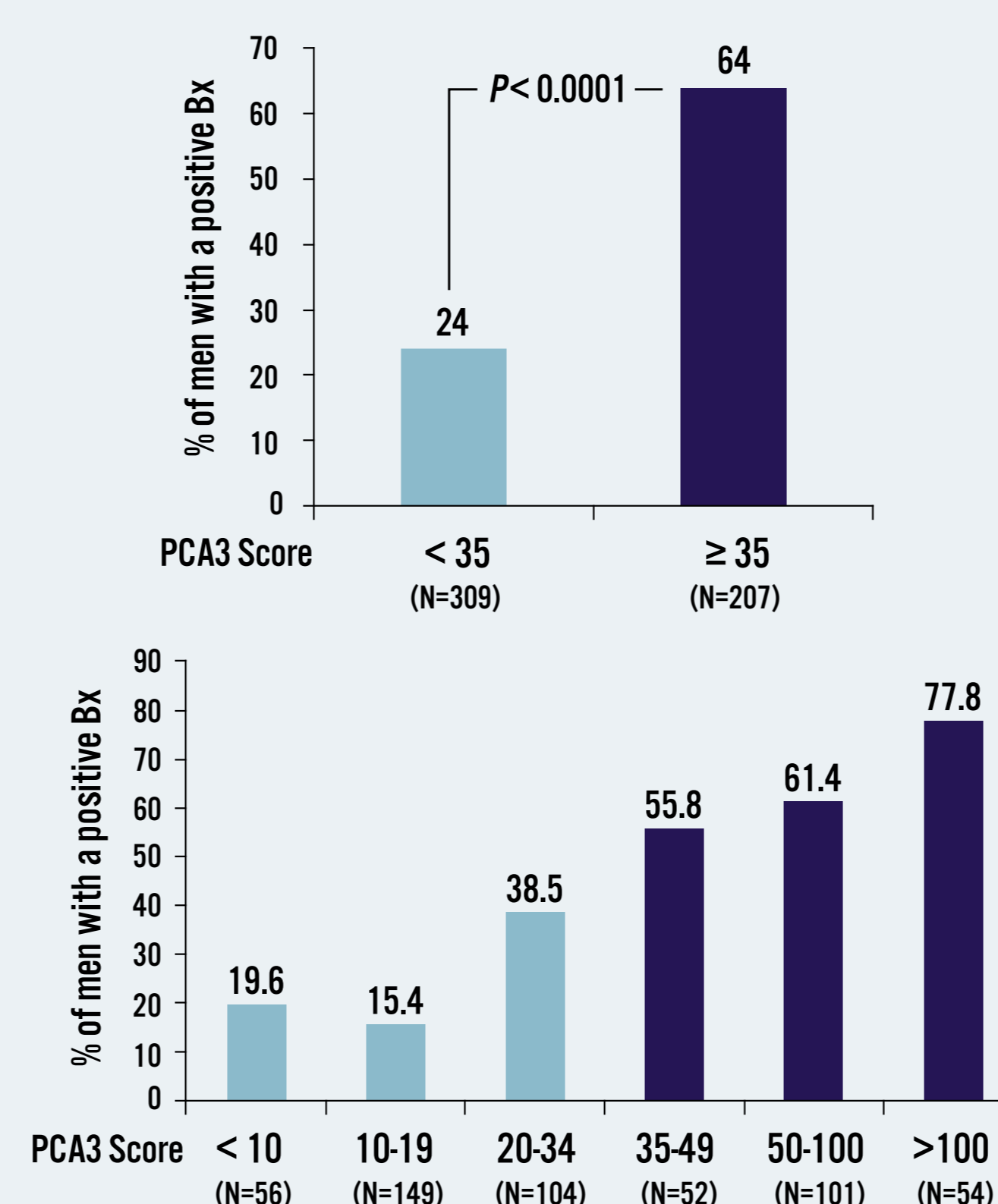


FIGURE 3: THE HIGHER THE PCA3 SCORE, THE GREATER THE PROBABILITY OF A POSITIVE Bx

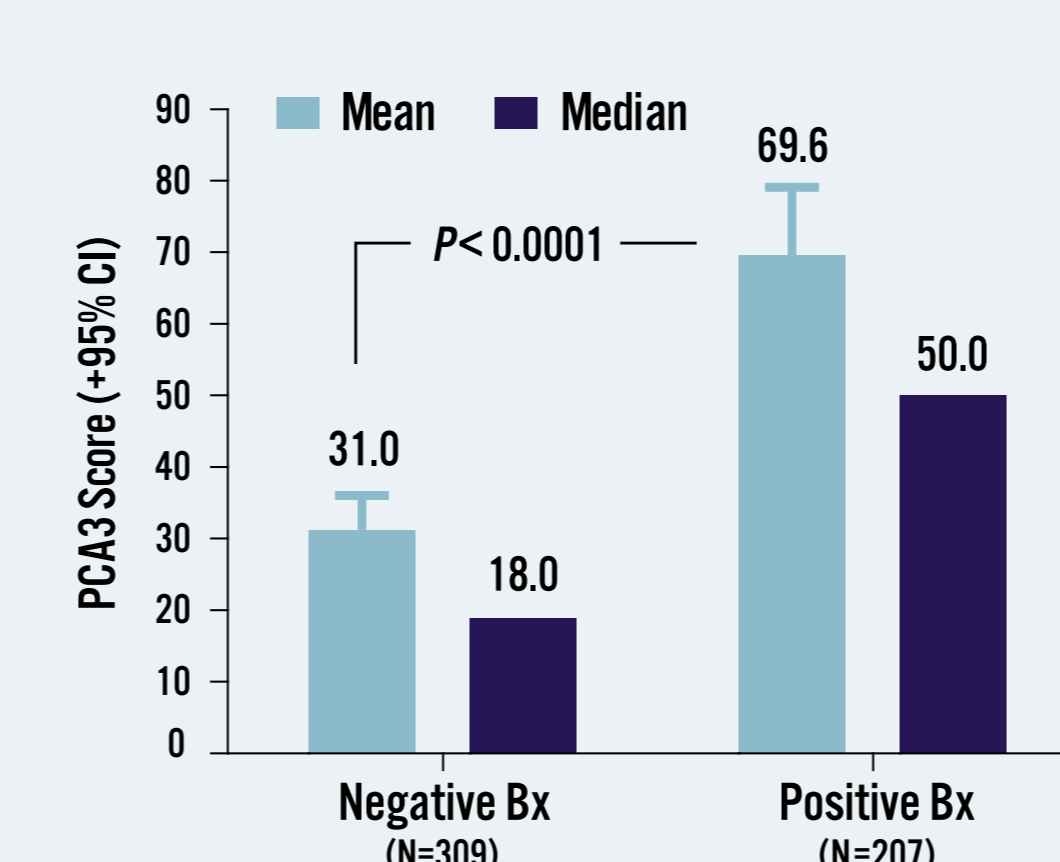


FIGURE 4: THE MEAN PCA3 SCORE WAS STATISTICALLY SIGNIFICANTLY HIGHER IN MEN WITH A POSITIVE Bx THAN IN MEN WITH A NEGATIVE Bx

### Impact of clinical variables on PCA3 Score

- The PCA3 Assay had similar performance characteristics (AUC ROC) in men
  - aged < 65 years vs.  $\geq 65$  years: 0.775 vs. 0.707
  - with a serum PSA  $\geq 2.5$ -4 ng/mL vs.  $\geq 4$ -10 ng/mL: 0.754 vs. 0.760

### Relationship between PCA3 Score and significance of cancer

- The mean PCA3 Score was statistically significantly higher in men with (Table 4)
  - Gleason score  $\geq 7$  vs. < 7
  - > 33% vs.  $\leq 33\%$  positive cores and
  - 'significant' vs. 'indolent' cancer

TABLE 4: THE MEAN PCA3 SCORE IS STATISTICALLY SIGNIFICANTLY HIGHER IN MEN WITH GLEASON SCORE  $\geq 7$  VS. < 7, > 33% VS.  $\leq 33\%$  POSITIVE CORES AND 'SIGNIFICANT' VS. 'INDOLENT' PCA

| PCA3 Score in:               | N   | Median | Mean | $\pm$ 95 CI | Pvalue** |
|------------------------------|-----|--------|------|-------------|----------|
| All men with a Bx+           | 207 | 50     | 69.6 | 59.5-79.7   | -        |
| Gleason score < 7            | 109 | 40     | 59.4 | 43.7-75.2   | <0.0001  |
| Gleason score $\geq 7$       | 98  | 72     | 80.9 | 68.7-93.0   |          |
| % positive cores $\leq 33\%$ | 122 | 39     | 61.8 | 46.5-77.0   | <0.0001  |
| % positive cores > 33%       | 84  | 78.5   | 80.6 | 69.3-92.0   |          |
| 'Indolent' PCA*              | 37  | 31     | 61.0 | 19.5-102.5  | 0.0016   |
| 'Significant' PCA            | 111 | 57     | 71.0 | 59.7-82.2   |          |

Bx+: positive Bx; CI: confidence interval; \*\* Wilcoxon Rank Sum test; \* indolent PCA defined as: clinical stage T1c, PSA density < 0.15, Gleason score Bx  $\leq 6$ , percent of positive cores  $\leq 33\%$

### Relationship between PCA3 Score and high-grade prostate intraepithelial neoplasia (HGPIN)

- The mean PCA3 Score was statistically significantly higher in men with HGPIN (81.9; median 66.5; N = 64) than in men without HGPIN (41.0; median 23;  $P = 0.0001$ ; N = 440)
- In men with a negative Bx, those with HGPIN also had a statistically significantly higher mean PCA3 Score (44.5; median 31; N = 24) than those without HGPIN (29.4; median 16.5;  $P = 0.0009$ ; N = 280)

### Characteristics of missed PCa

- When using a PCA3 Score cut-off of 35, 309 (60%) Bx could have been avoided while 22 PCa (11%) with a Gleason score  $\geq 7$  would have been missed (Table 5)
- When using a PCA3 Score cut-off of 20, 205 (40%) Bx could have been avoided while 5 PCa (2%) with a Gleason score  $\geq 7$  would have been missed (Table 5)

TABLE 5: CHARACTERISTICS OF MISSED PCA

| PCA3 Score cut-off         | 20     | 35       |
|----------------------------|--------|----------|
| All Gleason score $\geq 7$ | 5 (2%) | 22 (11%) |
| • Gleason score 7          | 5 (2%) | 20 (10%) |
| • Gleason score 8          | 0      | 1 (0.5%) |
| • Gleason score 9          | 0      | 1 (0.5%) |
| • Clinical stage T2        | 0      | 4 (2%)   |
| • % positive cores > 33%   | 2 (1%) | 9 (4%)   |

Total number of PCa: 207

### Multivariable analysis

- Inclusion of PCA3 into multivariable models increased their predictive accuracy by up to 5.5% (Table 6)

TABLE 6: MULTIVARIABLE LOGISTIC REGRESSION MODELS PREDICTING PRESENCE OF PCA AT INITIAL PROSTATE BIOPSY

| Variables                            | Multivariable models     |   |   |   |
|--------------------------------------|--------------------------|---|---|---|
|                                      | Base model <sup>1</sup>  | Base model <sup>1</sup> + continuous coded PCA3 | Base model <sup>1</sup> + PCA3 cut-off 17 | Base model <sup>1</sup> + PCA3 cut-off 35 |
| Age                                  | OR (95% CI); Pvalue      | OR (95% CI); Pvalue                             | OR (95% CI); Pvalue                       | OR (95% CI); Pvalue                       |
| DRE suspicious vs. unsuspecting      | 2.30 (1.38-3.86); 0.001  | 2.24 (1.32-3.80); 0.003                         | 2.10 (1.23-3.59); 0.006                   | 2.16 (1.25-3.72); <0.001                  |
| PSA                                  | 1.15 (1.04-1.28); 0.006  | 1.15 (1.03-1.28); 0.013                         | 1.14 (1.03-1.27); 0.016                   | 1.13 (1.01-1.26); 0.033                   |
| Prostate volume                      | 0.96 (0.95-0.98); <0.001 | 0.96 (0.95-0.98); <0.001                        | 0.97 (0.95-0.98); <0.001                  | 0.97 (0.95-0.98); <0.001                  |
| PCA3 Score                           | -                        | 1.01 (1.00-1.01); <0.001                        | 4.52 (2.66-7.69); <0.001                  | 4.38 (2.79-6.86); <0.001                  |
| Predictive accuracy                  | 0.737                    | 0.780   | 0.783                                     | 0.792                                     |
| Increment in predictive accuracy (%) | -                        | 4.3 [P=0.11]*                                   | 4.6 [P=0.08]*                             | 5.5 [P=0.03]*                             |

OR: odds ratio; CI: confidence interval; DRE: digital rectal examination; PSA: prostate specific antigen; PCA3: Prostate CAncer gene 3; <sup>1</sup>Base model: age, DRE, PSA, total prostate volume; \* Mantel Haenszel Test

## Conclusions

- These European data show that in men with a serum total PSA 2.5-10.0 ng/mL, an increasing PCA3 Score corresponds with an increasing probability of a positive initial Bx
- PCA3 was superior to serum total PSA, PSAD and % free PSA for predicting initial Bx result
- The clinical utility of the PCA3 Assay is independent of age and serum total PSA level
- The PCA3 Score may be indicative for aggressiveness of PCa and may aid in the selection of men to be managed with active surveillance
- The PCA3 Score is higher in men with HGPIN and may identify men at risk of developing PCa
- The PROGENSA<sup>®</sup> PCA3 Assay is a simple urine test that can be of additional help for urologists in counselling patients whether or not to perform an initial Bx

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