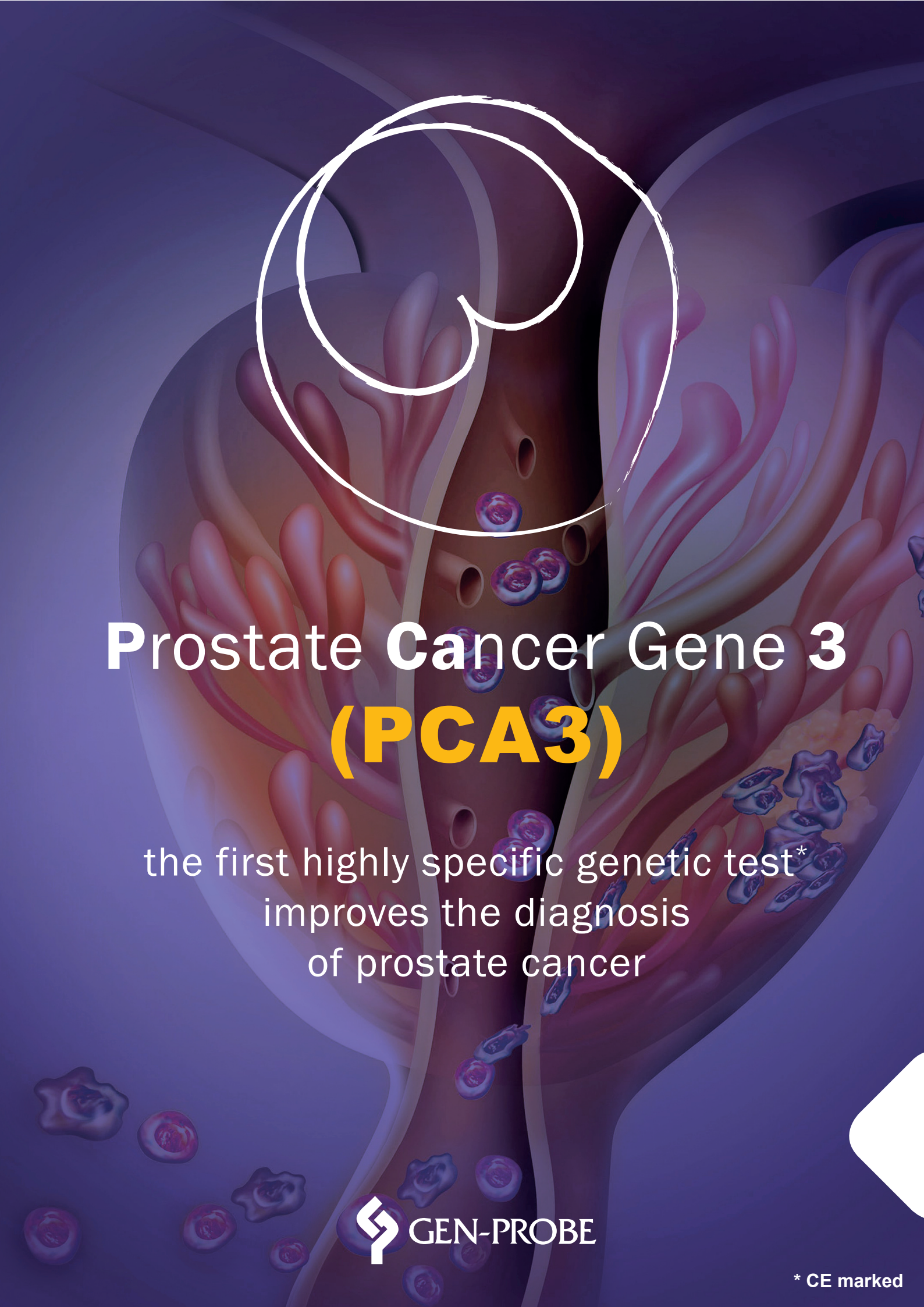


Prostate Cancer Gene 3 (PCA3)

the first highly specific genetic test*
improves the diagnosis
of prostate cancer

GEN-PROBE

* CE marked

Introduction

Prostate cancer (PCa) is the most commonly diagnosed malignancy and the second leading cause of cancer death in (American) men. One in 6 men will be diagnosed with PCa at some point in his life, but only one in 50 will die from PCa. In other words, the prevalence of PCa is very high, but the aggressiveness of the malignancy varies so much that more men will die with PCa than because of PCa.
In the diagnosis of PCa the challenge is to detect clinically significant PCa that still can be cured. In most patients with PCa, symptoms do not develop when the tumour is still localised. Currently the early detection of PCa relies primarily on the serum prostate specific antigen (PSA) concentration and digital rectal examination (DRE). The outcome of both tests can result in a prostate biopsy to confirm the diagnosis of PCa.

The dilemmas when using DRE, PSA and prostate biopsy in the diagnosis of prostate cancer

DRE dilemma

DRE is a standard tool in the diagnosis of PCa. Nevertheless, the exam by itself has a very low positive predictive value (PPV) in identifying PCa. In a study in 986 men undergoing biopsy for an abnormal / suspicious DRE with low PSA levels (< 4.0 ng/mL), only 8.8% of the patients had PCa [1]. Other studies have reported PPVs of 10-19% in this patient population [1]. The DRE reproducibility is poor and inter-examiner variability high [1].

PSA dilemma

PSA is a protein produced by the prostate and is not PCa-specific. Serum PSA levels increase as the prostate gland enlarges due to benign prostatic diseases such as lower urinary tract symptoms suggestive of benign prostatic hyperplasia (LUTS/BPH) and prostatitis. The European Randomized Study of Screening for Prostate Cancer (ERSPC) [2] and the Prostate Cancer Prevention Trial (PCPT) [3] have shown that no certain serum PSA level can accurately separate malignant from benign cases. Therefore, the PPV of serum PSA, even at a level of up to 10 ng/mL, is low (20-30%) [4,5].

Prostate biopsy dilemma

Transrectal ultrasound (TRUS)-guided biopsy provides the definitive diagnosis of PCa. However, because of the lack of specificity of DRE and serum PSA, an increasing number of men will undergo an unnecessary initial biopsy (around 65% for serum PSA levels between 4.0 ng/mL and 10 ng/mL) [5]. This number will continue to grow due to increased screening and use of lower serum PSA thresholds. In a recent survey among 139 urologists in Belgium, France, Germany, the Netherlands and the UK almost two thirds of respondents indicated at least 41% of initial biopsies were negative. Whereas for one in five of the respondents this percentage was even greater with at least 61% of negative biopsy results.

Since only a small fraction (around 1%) of total prostate tissue is obtained during biopsy, a negative result does not imply that the patient does not have PCa. Approximately 10-25% of men with PCa remain undiagnosed after a single sextant biopsy [5] and in one study 11% of men with a negative biopsy developed PCa within 7 years [6]. Therefore, an initial negative biopsy will frequently trigger repeat biopsies to confirm that PCa is not present.

The high percentage of initial negative and repeat biopsies will continue to increase health care costs while diminishing patients' quality of life. Biopsies induce anxiety because of the fear of having PCa and they cause discomfort and pain. The patient can also have complications after the procedure. The incidence of discomfort, pain and complications (haematuria, haematospermia and rectal bleeding) are shown in Table 1.

Table 1: Percent of patients reporting complications following a 6-12 core prostate biopsy (range reported in prospective studies involving at least 100 patients) [5-13]

COMPLICATION	% OF PATIENTS
Discomfort	34-70%
Pain	50-70%
Haematuria	10-74%
Haematospermia	10-78%
Rectal bleeding	1-40%

Complications requiring hospitalisation (such as severe haematuria or septicaemia) occur in approximately 1% of patients, despite antibiotics being administered prophylactically. One study showed that 1 in 10 patients would refuse repeat prostate biopsy or would require sedation / analgesia [6] which indicates that biopsies are indeed painful and bothersome for patients.

Taking all these factors into consideration, there is obviously a need for an additional diagnostic test that has an improved specificity for PCa and can better identify patients who will have a positive biopsy. This will reduce the number of initial and repeat biopsies.

Current dilemmas in the diagnosis of PCa

- An increased serum PSA concentration and an abnormal / suspicious DRE are both triggers to perform a prostate biopsy
- The PPV of both DRE (< 20%) and serum PSA (< 30% at levels below 10 ng/mL) is far from optimal
- Approximately two thirds of the initial biopsies are negative
- Since only approximately 1% of tissue is obtained during a biopsy, the fear that PCa may have been missed, when the initial biopsy is negative, can lead to repeat biopsies
- Prostate biopsy is not only costly but also causes distress, pain and discomfort for the patient and can lead to (sometimes severe) complications
- There is need for an additional diagnostic test to better predict biopsy outcome and reduce unnecessary biopsies

PCA3 and its role in improving the diagnosis of prostate cancer

Introduction to PCA3

The PCA3 Assay (Prostate Cancer Gene 3; in the past referred to as PCA3^{DD3} or DD3^{PCA3}) is the first genetic diagnostic test to help solve the mentioned clinical dilemmas associated with the diagnosis of PCa. In contrast to serum PSA, PCA3 is not only prostate-specific but also PCa-specific (Figure 1) [14]. Messenger RNA (mRNA) of the PCA3 gene is highly over-expressed (median 66-fold) in > 95% of PCa tissue compared to normal or benign prostate tissue of the same patients [14,15].

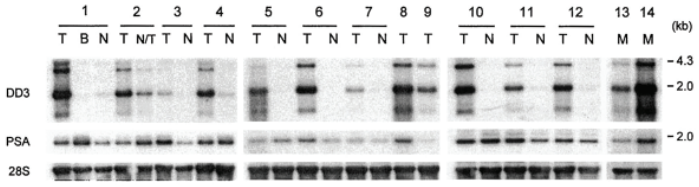


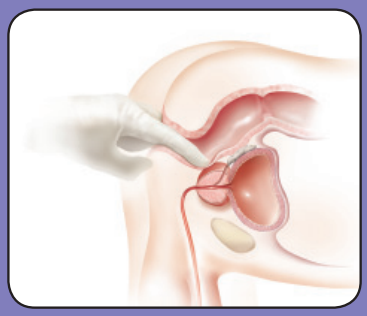
Figure 1: PCA3 (DD3) mRNA is over-expressed in prostatic tumour (T and M) compared to benign (B) or normal (N) tissue, in contrast to PSA mRNA [14]

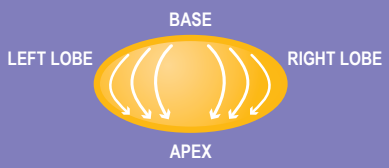
Using the PCA3 Assay in clinical practice

The Gen-Probe® PCA3 Assay [16] detects the presence of PCA3 mRNA from urine (Figure 2):

I. Perform a DRE (3 strokes per lobe) to release a sufficient number of prostate cells into the urine
II. After the DRE, collect 20-30 mL first-catch urine from the patient
III. Transfer 2 mL of urine to the transport tube
IV. Store the transport tube with the urine at or below 8ºC (may be frozen) and ship the transport tube as soon as possible to the laboratory.

Note: The transport tubes can be obtained from a PCA3 testing laboratory.





DRE
(3 STROKES PER LOBE)

FIRST CATCH URINE SPECIMEN
(20-30 mL)

TRANSPORT TUBE WITH URINE (2 mL)
SENT IN COLD PACK (≤ 8°C)
DIRECTLY TO LABORATORY

Figure 2: The PCA3 urine specimen collection procedure
Apply pressure on the prostate, enough to depress the surface approximately 1 cm, from the base to the apex and from the lateral to the median line for each lobe and repeat this 3 times

Using the PCA3 Assay result for improving the diagnosis of prostate cancer

Calculation of the PCA3 Score

In order to be sure that a sufficient number of prostate cells / prostate RNA is present in the urine specimen and in order to normalise the PCA3 amount, the laboratory also measures mRNA of PSA in the urine sample. Using transcription-mediated amplification technology, PCA3 and PSA mRNA molecules are amplified and the PCA3 Score is calculated.

The PCA3 Score = 1000 x [mRNA PCA3] / [mRNA PSA].

PCA3 Score in men with serum PSA ≥ 2.5 ng/mL and ≥ 1 negative prostate biopsy

The PCA3 Score was determined in 233 men (mean age 64 years) with serum PSA ≥ 2.5 ng/mL (mean 7.4 ng/mL) and at least one negative biopsy [17]. In 226 urine samples (97%), there was sufficient mRNA present for analysis. Repeat biopsies were positive for 60 men (27%). It appeared that the higher the PCA3 Score, the higher the percentage of men with a positive biopsy (Figure 3).
A patient with a PCA3 Score ≥ 35 has a high probability of having PCa and repeat biopsy is advisable. If the outcome is negative (PCA3 Score < 35), the patient has a lower probability of having PCa and repeat biopsy might be delayed with active surveillance / follow-up of the patient including monitoring of serum PSA.

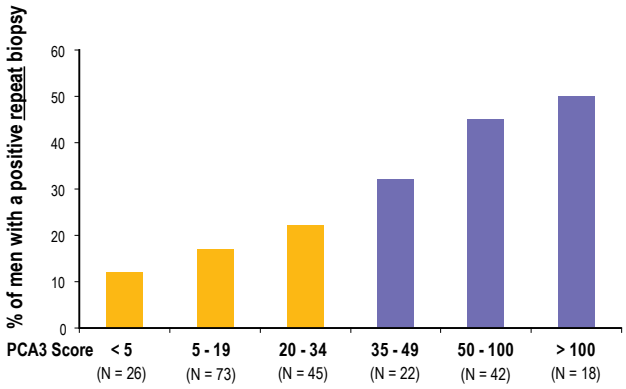


Figure 3: The higher the PCA3 Score, the higher the percentage of men with a positive repeat biopsy [17]

PCA3 Score distinguishes normal men and men with LUTS/BPH from men with PCa

The PCA3 Score also discriminates normal men (aged < 45 years with no known PCa risk factors) from men with LUTS/BPH, untreated PCa and PCa treated with radical prostatectomy. There was no overlap in the 95% confidence intervals (CIs) between the groups ($P < 0.01$) (Figure 4) [18].

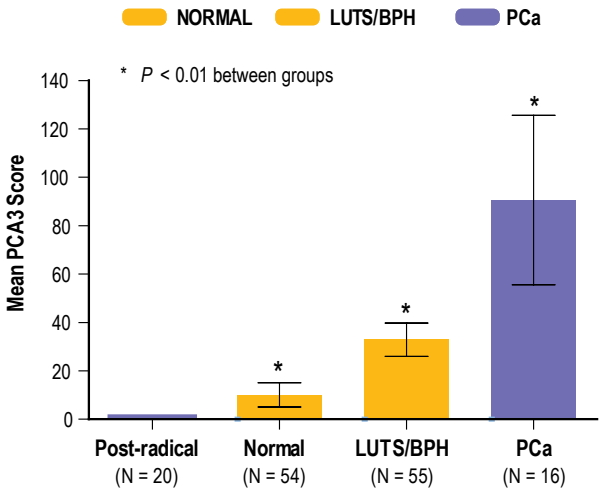


Figure 4: The PCA3 Score seems to be able to discriminate between normal / benign vs. malignant prostate tissue[18]

PCA3 Score in men scheduled for initial or repeat prostate biopsy

The PCA3 Score was also determined in 529 men who were scheduled for (initial or repeat) biopsy or prostatectomy [19]. In total 180 men (34%) had a positive biopsy. Also in these men it was shown that the higher the mean PCA3 Score, the higher the percentage of patients with a positive biopsy (Figure 5).

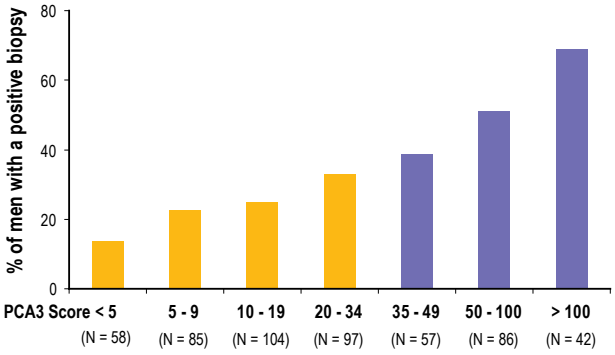


Figure 5: The higher the PCA3 Score, the higher the percentage of men with a positive biopsy [19]

PCA3 Score is independent of prostate volume

In the same population of men scheduled for (initial or repeat) biopsy or prostatectomy, the mean serum PSA concentration increased with larger prostate volumes whereas the mean PCA3 Score was not influenced by prostate volume (Figure 6) [20].

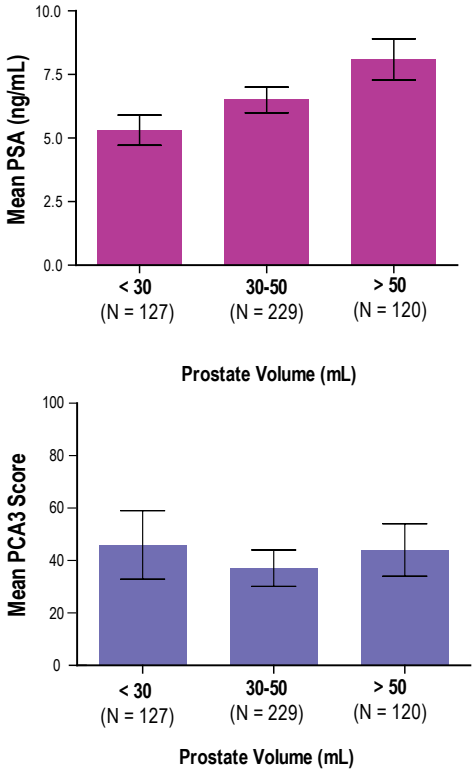


Figure 6: Serum PSA (mean ± 95% CI) clearly increases with prostate volume; the PCA3 Score (mean ± 95% CI) is independent of prostate volume [20]

The PCA3 Assay: a highly specific genetic test to improve the diagnosis of prostate cancer

- The PCA3 Assay is a genetic test which is highly PCa-specific
- The PCA3 Assay uses urine to measure the PCA3 and PSA mRNA concentration and calculate the PCA3 Score
- The PCA3 Score is not influenced by prostate volume
- The probability of a positive biopsy increases with higher PCA3 Scores
- A PCA3 Score of ≥ 35 in men with a PSA ≥ 2.5 ng/mL and at least one negative biopsy may indicate that repeat biopsy is advisable;
  for those with a PCA3 Score < 35, biopsy might be delayed based on active surveillance / follow-up of the patient
- The PCA3 Score may also be used to determine the need for an initial biopsy in men with an elevated serum PSA (≥ 2 ng/mL) or with a low serum PSA but suspicious DRE; current research in these patient populations is addressing this question
- The PCA3 Assay can identify patients who have a high probability of having PCa
- The PCA3 Assay is a useful test for improving the diagnosis of PCa and reducing the number of unnecessary biopsies

Reference List

[1] Bozeman CB, Carver BS, Caldito G, Venable DD, Eastham JA. Prostate cancer in patients with an abnormal digital rectal examination and serum prostate-specific antigen less than 4.0 ng/mL. Urology 2005;66: 803-7.

[2] Postma R, Schröder FH. Screening for prostate cancer. Eur J Cancer 2005;41: 825-33.

[3] Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL et al. Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. N Engl J Med 2004;350: 2239-46.

[4] Vollmer RT. Predictive probability of serum prostate-specific antigen for prostate cancer: an approach using Bayes rule. Am J Clin Pathol 2006;125: 336-42.

[5] Seitz C, Palermo S, Djavan B. Prostate biopsy. Minerva Urol Nefrol 2003;55: 205-18.

[6] Raja J, Ramachandran N, Munneke G, Patel U. Current status of transrectal ultrasound-guided prostate biopsy in the diagnosis of prostate cancer. Clin Radiol 2006;61: 142-53.

[7] Autorino R, De Sio M, Di Lorenzo G, Damiano R, Perdonà S, Cindolo L et al. How to decrease pain during transrectal ultrasound guided prostate biopsy: a look at the literature. J Urol 2005;174: 2091-7.

[8] Raaijmakers R, Kirkels WJ, Roobol MJ, Wildhagen MF, Schröder FH. Complication rates and risk factors of 5802 transrectal ultrasound-guided sextant biopsies of the prostate within a population-based screening program. Urology 2002;60: 826-30.

[9] Ghani KR, Dundas D, Patel U. Bleeding after transrectal ultrasonography-guided prostate biopsy: a study of 7-day morbidity after a six-, eight- and 12-core biopsy protocol. BJU Int 2004;94: 1014-20.

[10] Peyromaure M, Ravery V, Messas A, Toublanc M, Boccon-Gibod L, Boccon-Gibod L. Pain and morbidity of an extensive prostate 10-biopsy protocol: a prospective study in 289 patients. J Urol 2002;167: 218-21.

[11] Mäkinen T, Auvinen A, Hakama M, Stenman UH, Tammela TL. Acceptability and complications of prostate biopsy in population-based PSA screening versus routine clinical practice: a prospective, controlled study. Urology 2002;60: 846-50.

[12] Djavan B, Waldert M, Zlotta A, Dobronski P, Seitz C, Remzi M et al. Safety and morbidity of first and repeat transrectal ultrasound guided prostate needle biopsies: results of a prospective European prostate cancer detection study. J Urol 2001;166: 856-60.

[13] Naughton CK, Ornstein DK, Smith DS, Catalona WJ. Pain and morbidity of transrectal ultrasound guided prostate biopsy: a prospective randomized trial of 6 versus 12 cores. J Urol 2000;163: 168-71.

[14] Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res 1999;59: 5975-9.

[15] Hessels D, Klein Gunnewiek JM, van Oort I, Karthaus HF, van Leenders GJ, van Balken B et al. DD3^{PCA3}-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003;44: 8-16.

[16] Groskopf J, Aubin SM, Deras IL, Blase A, Bodrug S, Clark C et al. APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Clin Chem 2006;52: 1089-95.

[17] Marks LS, Fradet Y, Deras IL, Blase A, Mathis J, Aubin SMJ et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology 2006: *Submitted*.

[18] Groskopf J, Aubin SM, Deras IL, Blase A, Bodrug S, Brentano S et al. Specificity of the APTIMA® PCA3 assay for prostate cancer. Eur Urol Suppl 2006;5: 240 (abs. 870). Poster presented at EAU 2006.

[19] Gen-Probe Incorporated data on file; Label CE Mark. 2006.

[20] Marks LS, Aubin SMJ, Deras IL, Brentano S, Blase A, Bodrug S et al. Specificity of the APTIMA® PCA3 assay for prostate cancer. J Urol 2006;175(4 Suppl): 175-6 (abs. 540). Poster presented at AUA 2006.