The role of the PCA3 assay in predicting prostate biopsy outcome in a South African setting

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Abstract

OBJECTIVES

• To evaluate the investigational role, ideal threshold and indications of the Prostate CAncer gene 3 (PCA3) assay in a South African context.
• To better define the universality of the above marker since this is the pioneer study on the continent of Africa.

PATIENTS AND METHODS

• We prospectively evaluated 105 consecutive South African men referred for a prostate biopsy at two tertiary centres in the capital city, Pretoria.
• Sequentially, PSA levels and post DRE urine samples were taken within 24 h before prostate biopsy.
• The urine specimen was tested using the PROGENSA^{TM} PCA3 assay and a score was generated as (PCA3 mRNA/PSAmRNA) x 1000.
• The performance of this assay in predicting biopsy outcome was assessed, and compared with that of serum PSA.

RESULTS

• Median patient age was 67 years with a positive biopsy incidence of 42.9%.
• The higher the PCA3 score the greater the probability of a positive biopsy (P = 0.003).
• This score performed independently of prostatic volume (P = 0.3889) or the presence of a concurrent primary malignancy (P = 0.804).
• A threshold of 60 revealed a positive predictive value of 60% with an odds ratio of 4, whereas setting a limit of 35 revealed a positive predictive value of 54% and odds ratio of 3.5.
• Using receiver operating characteristics for overall performance comparison, the PSA level (area under the curve 0.844) performed better than the PCA3 score (area under the curve 0.705).

CONCLUSION

• PCA3 assay has shown consistency and performed in line with previous studies but it did not surpass serum PSA in this population.
• A PCA3 assay threshold of 60 performed better than the conventional limit of 35.

• This assay may have a potential niche in a certain subset of South African men that includes patients with larger glands, previous negative biopsies and altered baseline PSA levels.

KEYWORDS
biomarker, Prostate CAncer gene 3, prostate histology, prostate-specific antigen, South Africa

Introduction

Prostate cancer (CaP) is a common entity worldwide, with a higher reported incidence amongst South African Black men (8.5%) when compared to South African Caucasian men (3.7%) [1]. However, the prevalence of prostate specific antigen (PSA)-detected CaP in South Africa has been described as at least 'similar' amongst both racial groups [1,2].

In confirming the presence of CaP, the current standard trigger for a prostate biopsy consists of an abnormal digital rectal examination (DRE) and, or an elevated total serum PSA level [3]. In reality, both the above parameters have a poor positive predictive value (PPV) in detecting CaP, with a meta-analysis revealing a PPV range for DRE and PSA at 5-33 % and 17-57% respectively [4].

The PSA level has other well described limitations, with false elevations in common non-cancerous states, including prostatitis and benign prostatic hyperplasia (BPH) [5]. A recent French study revealed that this level also expresses a seasonal variation, with a 23% higher possibility of having a PSA level above 3ng/ml, if screening was carried out in the summer months [6].

Some of the above drawbacks of the PSA level have led to the development of a novel marker, the Prostate CAncer gene 3 (PCA3) assay. Since having first been described to have a significant overexpression in 53 of 56 prostatic tumours [7], the PCA3 (formerly known as DD3) assay has progressed into a promising biomarker with great potential for use in the clinical scenario.

A comprehensive review of the first 11 studies (2723 men) assessing the PCA3 assay in Western countries forecast a significant future in its role as a predictor of prostate biopsy outcome [8].

We therefore deemed it necessary to assess this biomarker in a more demographically suitable context. Being the first study of this nature in an African setting, an added objective would be to better define the universality of the PCA3 assay.

We thus set out to evaluate this assay in the prediction of prostate biopsy outcome among South African men, at two major tertiary referral centres in Pretoria.

Patients and Methods

We prospectively assessed the role of the PCA3 assay amongst consecutive men who were already scheduled for a prostate biopsy. Ethics approval (protocol number: 60/2009) had been attained at the University of Pretoria, Ethics Committee and the study was registered with the South African National Clinical Trial Register and the Department of Health, URL: www.sanctr.gov, with trial number: DOH-27-0609-2892.

Written consent was obtained from all patients, with each patient being assigned a unique study number. The study was performed at the Steve Biko Academic and Kalafong Hospitals, based in Pretoria. Patients with indwelling urethral and supra-pubic catheters were excluded. All age and race groups were considered, irrespective of the referral PSA level. Patients scheduled for the first or repeat prostate biopsy were included. Over a period of eight months (ending in February 2010) a total of 107 men were assessed.

Venous blood was taken for the study serum PSA level, which was measured using the WHO calibrated Beckman Coulter Access Hybritech ® system. Thereafter, a DRE as described by Groskopf et al. [9] was performed, followed by collection of a first-catch urine specimen.

A unique study code was assigned to each urine sample and the specimen was assessed to quantify PCA3 and PSA mRNA concentrations using the PROGENSA^{TM} PCA3 assay. A PCA3 score was generated as (PCA3 mRNA/PSA mRNA) x 1000.

Blood and urine specimen collection was always obtained in the above order and was both collected within 24h prior to the scheduled prostate biopsy.

Antibiotic prophylaxis was administered prior to biopsy. Using a Trans Rectal Ultrasound (TRUS) probe, the prostate volume was always assessed using the height measurement obtained trans-axially as recommended by Park et al. [10]. TRUS biopsies of the prostate gland were then performed using a standardised systematic 13-core 5-region biopsy method [11].

The physician performing the biopsies was blinded to the study PSA level and PCA3 score. For standardisation purposes, every DRE, first-catch urine specimen collection, TRUS volume assessment and prostate biopsy was performed by the same physician.

The serum PSA, urinary PCA3 assay and prostatic histological assessment was always performed by the consistent respective group of pathologists in the same relevant laboratory. All the above staff members were blinded to the patient's study details.

The final histological outcome for cancer was then compared and contrasted to each of the above predictive parameters.

Results

Among 107 subjects, 105 urine sediment samples had sufficient concentrations of PCA3 and PSA mRNA to generate a PCA3 score, resulting in an informative rate of 98.1%. The demographic details of the patient population are listed in Table 1. The mean age was 67 yr. Majority of the patients were Black (68.6%), with fewer Caucasians (25.7%) and the remainder belonging to other racial denominations (5.7%). A positive family history of CaP was present in only 4.6% of all patients. Most men (81.9%) were scheduled for their first biopsy. Overall, a wide range of risk stratification for cancerous disease had been observed, with 48.6% of patients having a suspicious DRE and 27.6 % of patients having a study PSA less than 4ng/ml. Histology reports revealed the presence of CaP in 42.9% of patients. In a review of the Gleason sum amongst the cancer cohort, 28/44 patients scored 6 or less, 12/44 patients scored 7 and the reminder 4/44 patients scored 8 or more. Amongst the non-cancerous group, BPH was the commonest (80%) histological finding.

An increase in the PCA3 score was found to be associated with an increase in the incidence of CaP (p=0.003) (Figure 1). By implementing the suggested cut-off value of 35 [9], a sensitivity of 77.7% and specificity of 50% was reached, in contrast to a higher cut-off of 60, which yielded an overall sensitivity and specificity of 68.9% and 66.7%, respectively (Table 2). Black patients presented with higher PCA3 scores ($p < 0.05$) and PSA levels ($p < 0.05$) when compared to their Caucasian counterparts. However, the predictability of the PCA3 score amongst Black men was less impressive than that of Caucasian men (Table 3). These two groups also performed differently when applying different cut off points, with the best overall predictability (specificity of 90.0%, sensitivity of 71.4%) being reached when applying a cut-off of 60 amongst Caucasian men.

The receiver operating characteristic (ROC) curve analysis yielded an AUC of 0.7054 (95% confidence interval (CI): 0.599 to 0.812) for the PCA3 score. Overall, the PSA level performed better in this population, achieving an AUC of 0.8443 (95% CI: 0.764 to 0.910) (Figure 2). The PCA3 score performed independent of gland volume (p=0.3889) (Figure 3a), as opposed to the PSA level, which revealed a significant proportional correlation (p=0.0428) (Figure 3b).The sum of the PCA3 score and PSA level (AUC of 0.8306 (95 % CI: 0.743 to 0.895)) in this population did not perform better than the PSA level alone (Figure 4).

When comparing the performance of the PCA3 assay amongst various PSA level ranges, the PCA3 performed best in the 'PSA gray zone' (4 to 10 ng/ml) reaching a specificity and sensitivity of 64.7% and 85.7% respectively (Table 4).

Amongst the cohort, 29 (27.6%) men were observed to have a 'study' PSA level < 4 ng/mL (Table 5), with 11/29 being found to have an associated suspicious finding on DRE. The remainder 18/29 men had a normal DRE, but biopsies were performed even though their 'study' PSA level was < 4 ng/mL, since the physician performing the biopsy was blinded to the 'study' PSA result, and these 18/29 men were already scheduled for a biopsy based on their previous referral findings alone. This group thus represents the 'low risk' segment of the study population as they presented with a normal DRE, PSA level < 4 ng/mL (on day of biopsy) and absence of CaP on histology. The diagnostic ability of PSA (ROC AUC of 0.8550 (95% CI: 0.683 to 0.961)) was better than that of PCA3 (ROC AUC of 0.8100 (95% CI: 0.603 to 0.920)) in this PSA range group. Applying a PCA3 cut off point of 60, a sensitivity of 66% and a specificity of 70.7% was achieved within this subset. CaP was not present if a PSA level <4ng/ml was attained in combination with a normal DRE finding or a PCA3 score <60 (table 5).

When assessing the repeat biopsy group (n=19), the predictability of the PSA level (ROC AUC of 0.700 (95% CI: 0.435 to 0.874)) performed better than that of PCA3 (ROC AUC of 0.575 (95% CI: 0.335 to 0.797)). However, using a cut-off of 60, the PCA3 assay (sensitivity of 50%, specificity of 75%) performed better than the PSA level at a cut off of 4 ng/ml (sensitivity of 33%, specificity of 75%) in this group. A sensitivity of 75% and specificity of 46.6% was revealed if the conventional cut-off point of 35 for PCA3 assay was applied in this subset.

Since both the PSA level and the PCA3 score values were skewed to the right (non-normal), a non-parametric Kruskal-Wallis test was used to compare both values across the Gleason sum. There was evidence of an increase in the PSA level with an increase in the Gleason sum (p=0.057). The median PSA level was 21.57 ng/ml for a Gleason sum of 5 or less, 27.52 ng/ml for Gleason sum of 6 and 50.01 ng/ml for a Gleason sum of 7 or more, respectively. The relationship of the PCA3 score to the Gleason sum was not found to be systematic (p=0.111). With a median PCA3 score level of 174 for a Gleason sum of 5 or less, 72 for Gleason sum of 6 and 87 for a Gleason sum of 7 or more, respectively.

The presence of a concurrent malignancy (n=6) at the time of urine sediment collection did not significantly affect the overall AUC of this diagnostic assay (p=0.804). These associated malignancies included; liposarcoma (spermatic cord) [12], high grade squamous epithelial lesion (penis), paraganglionoma (bladder), malignant melanoma (skin) and the remaining 2/6 patients with transitional cell carcinoma (bladder).

No complications were encountered at follow-up.

Discussion

A direct comparison amongst this cohort has shown the PSA level to be a better predictor than the PCA3 assay across the range. The former marker in isolation has even proven to be superior to the cumulative sum (PSA and PCA3) of both the above contemporaries. In contrast however, Wang et al. [13] has shown this sum to perform better than the PSA level alone.

We have observed an acceptable informative rate (98.1%) in line with the initial 11 study ranges of 79 % to 100% [8].As an isolated biomarker, the PCA3 assay performance was 'good' with an overall AUC of 0.7054 (95% CI: 0.599 to 0.812). This finding is remarkable as it closely resembles the first study performed in 2003 evaluating a similar sample size of Dutch men (n=108), where an AUC of 0.717 (95% CI: 0.58 to 0.85) was observed [14]. The consistency and universality of the PCA3 assay is convincing, since the above similarity in performance assessment was attained on a different continent, evaluating a different population, almost seven years later.

Although much variation has been observed in the PCA3 assay performance with the application of different cut-offs, we observed the cut-off point of 60 to perform better than the conventional point of 35.

The inter-racial inconsistency of the PCA3 assay may be due to the added difference of risk stratification amongst both groups, since Black men had higher PCA3 scores and PSA levels at presentation, when compared to the Caucasian subset. This advanced cancer risk among South African Black men has been previously reported, with Heyns et al. [1] showing a higher percentage of an abnormal DRE and elevated PSA level amongst Black men when compared alongside other race groups.

In contrast to the PSA level, the PCA3 score performed independent of prostate gland volume and thus may prove useful in assessing men with larger glands. This finding of volume independence has been echoed in two previous studies which assessed European [15] and North American men [16].

Evidence of linear correlation of PSA with the Gleason sum was present. However, this was not the case with the corresponding PCA3 score, but Haese et al.[15] have previously shown the PCA3 assay to perform as a predictor of cancer severity in their series. A larger data set would prove beneficial to confirm or refute the above relationship in our setting.

When assessing the PCA3 specificity across PSA ranges, the highest specificity (64.7%) of PCA3 was amongst patients in the 'PSA gray zone'. The potential role of the PCA3 assay in this 'enigmatic' zone cannot be overemphasized, since it is within this range group that the urologist and pathologist commonly find themselves in a 'stalemate' situation. According to the literature, the specificity of the PCA3 assay in this 'PSA gray zone' has been favourable

but not constant, with values ranging from 71% [16] to 91% [17] in this PSA range. However, the finding of the PCA3 assay specificity performing best within the ' PSA gray zone' had been previously observed amongst a European cohort [15]. The above finding does prove that an essential role for the PCA3 assay does indeed lie in this 'PSA grey zone'.

When reviewing men in the lower PSA range group, none of the patients with a PSA level <4ng/ml associated with a PCA3 score < 60 (n=17), were observed to have CaP. Their inclusion in this study allows for representation of the 'true negatives' in this population group, thus allowing a widespread evaluation of the PCA3 assay across the risk stratification profile.

Combined with a PSA level <4 ng/ml, the PCA3 cut off point of 60 performed better than the point of 35, in identifying the 'low risk' group (table 5). Thus, the presence of a normal DRE, combined with a PCA3 score below 60 and a PSA level below 4ng/ml excluded the presence of underlying CaP in this study. Therefore, the PCA3 assay may also be advocated for use in combination with other parameters to better define the 'low risk' group in our population.

Another unique feature in this study is the impressive performance of the PSA level (AUC of 0.8443). Such an impressive AUC for PSA was not reported in the previous studies evaluating and comparing the PSA level with the PCA3 assay.

The *following* is a plausible explanation for the higher comparative performance of the PSA level in this study;

As the title suggests, we set out to assess the PCA3 assay in a truly representative 'local setting' and have included consecutive men scheduled for a prostate biopsy, irrespective of the PSA level. Some of these initial studies reviewed, [8] assessed men at certain PSA cut off levels. The PSA performance here would therefore be superior since 49.5% of this cohort had a PSA level > 10 ng/ml.

The fact that Black men comprised the largest racial denomination (68.6%) in this cohort had also influenced the PSA diagnostic accuracy, since PSA performs better amongst the higher risk stratification group [18], and South African Black men have been shown to have an advance cancer risk on presentation [1]. Amongst the positive biopsy group, a considerable amount had intermediate to high grade cancer, with Gleason sum scores greater than 6 in 36.4% of cases.

Since the referral PSA levels were taken at different locations, all at different time intervals we decided to repeat the PSA level within 24h prior to biopsy. Patients underwent biopsies regardless of this repeated PSA level. This repeated sample was assessed as the 'study' PSA level. The improvement in the diagnostic yield of a repeated PSA sample has been well established [19], and had influenced the diagnostic ability of the PSA in this scenario.

Absolute PSA levels were always used. This increased the specificity of the test, with Heyns et al. showing a specificity of 100% for absolute PSA levels above 200 ng/ml in a South African series [18].

The multicentre and inter-lab PSA variance factor was absent in this study, as all samples were analysed in the same laboratory within the same fashion, by a constant team of chemical pathologists.

None of the patients had reported being on any PSA baseline altering agents prior to biopsy, and a DRE was never performed prior to veni-punture for PSA, thus further increasing its diagnostic efficacy.

When assessing the repeat biopsy group, the performance interpretation of the PCA3 assay is to be done with caution, since they were only represented by a small number of patients (n=19) in this study. However, Deras et al. [16] has shown the PCA3 assay to perform superior to that of the PSA level in the repeat biopsy cohort, and has shown this marker to perform independent of a history of previous biopsies.

With this being the pioneer study on the continent of Africa, a larger trial with a direct inter-racial comparison of patients being corrected for their respective pre-biopsy risk amongst different racial groups will be needed in the future. The ideal cut-off points and their respective implications can also be better defined if a greater sample size is assessed. A further evaluation of the repeat biopsy group is also needed to confirm the PCA3 assays utility in this crucial subset of our population.

In conclusion, the PCA3 assay performance has shown to be consistent with previous studies, thus supporting its claim to universality. It has proven to function independent of prostatic volume. This assay has also been observed to perform independent of the presence of a concurrent primary malignancy. Although proven to be a ‘good’ biomarker, we have not witnessed it to be superior to the serum PSA level across the risk spectrum in this population.

The PCA3 assay could establish a significant role amongst a certain subset of South African men. This subset includes those anxious patients remaining in the ‘PSA gray zone’, patients with larger glands, previous negative biopsies, those being incorporated into risk nomograms, men with altered baseline PSA levels and those informed men who request it.

Tables and their legends

Parameter	**Number (%)**
Age (y)	
Median	**67**
Range	**35-89**
Race	
Black	**72 (68.6%)**
Caucasian	**27 (25.7%)**
Other	**6 (5.7%)**
History of previous biopsy	
No	**86 (81.9%)**
Yes (one or more)	**19 (18.1%)**
Family History of Prostate Cancer	
Yes	**5 (4.8%)**
No	**93 (88.6%)**
Other Cancer	**7 (6.6%)**
Men with serum PSA:	
0-4ng/ml	**29 (27.6%)**
>4-10ng/ml	**24 (22.9%)**
>10ng/ml	**52 (49.5%)**
Digital Rectal Examination	
Suspicious	**51 (48.6%)**
Not Suspicious	**54 (51.4%)**
Prostate Volume (ml)	
Median	**31.1**
Prostatic Biopsy : Histological Outcome	
Cancer	**45 (42.9%)**
Non-Cancerous condition	**60 (57.1%)**

Table 1. Demographic details for the 105 South African men.

Table 2. The PCA3 assay performance across various PCA3 assay score cut-off points.

PCA3 assay cut-off point	Sensitivity(%)	Specificity(%)	NPV(%)	PPV(%)	Odds Ratio (95% CI)
10	91.1	16.6	71	45	2.05 (0.599 - 7.020)
35	77.7	50.0	75	54	3.5 (1.472 - 8.321)
60	68.9	66.7	73	60	4.0 (1.762 - 9.081)

Abbreviations: PCA3 = Prostate CAncer gene 3; NPV = Negative predictive value; PPV = Positive predictive value; CI = Confidence interval

Table 3. The inter-racial comparison of the PCA3 assay's performance.

	Black (n= 72)	**Caucasian (n=27)**
PCA3 ROC AUC	0.645	0.804
Confidence Interval	0.508-0.773	0.577-1.00
Using 35 as cut off		
Sensitivity (%)	77.1	71.4
Specificity (%)	35.1	75.0
Using 60 as a cut off		
Sensitivity (%)	65.7	71.4
Specificity (%)	54.1	90.0

Abbreviations: n = patient number; ROC = receiver operating characteristics; AUC = Area under curve

Table 4. A comparison of the PCA3 assay performance within specific PSA score ranges, with the corresponding patient number in brackets.

PSA range (n)	PCA3 Specificity (%)	PCA3 Sensitivity (%)	p value
0-4 (29)	48	100	0.07
>4-10 (24)	64.7	85.7	0.025
>10 (52)	38.8	73.5	0.356

Abbreviations: PCA3 = prostate cancer gene 3; PSA = prostate-specific antigen

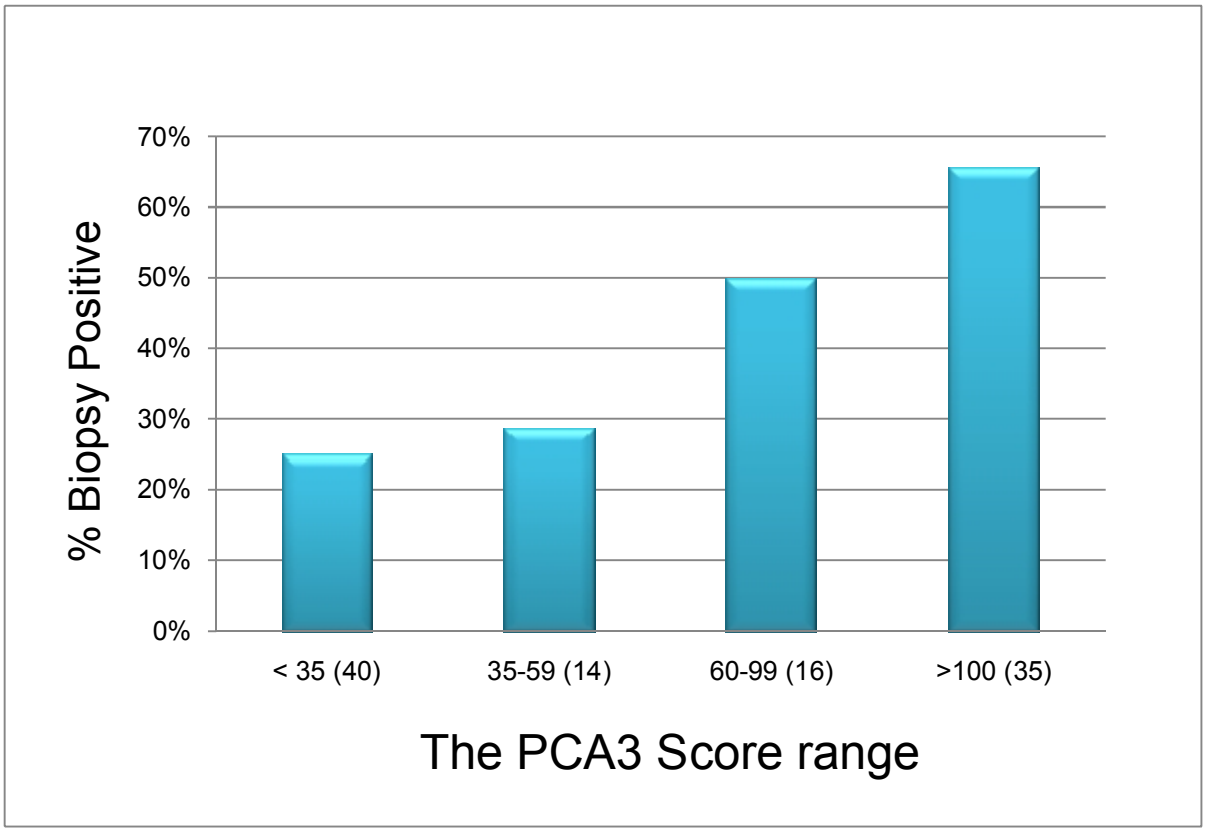
Table 5. Breakdown of the cohort with PSA <4ng/mL (n=29) combined with DRE findings and PCA3 score, contrasted against respective histological outcome.

PSA <4 ng/mL with,	Patient number	CaP on histology	No CaP detected on histology
Suspicious DRE	11	4	7
Normal DRE	18	0	18
PCA3 > 35	17	4	13
PCA3 < 35	12	0	12
PCA3 > 60	12	4	8
PCA3 < 60	17	0	17

Abbreviations: DRE= digital rectal examination; PCA3 = Prostate CAncer gene 3; CaP= Prostate Cancer

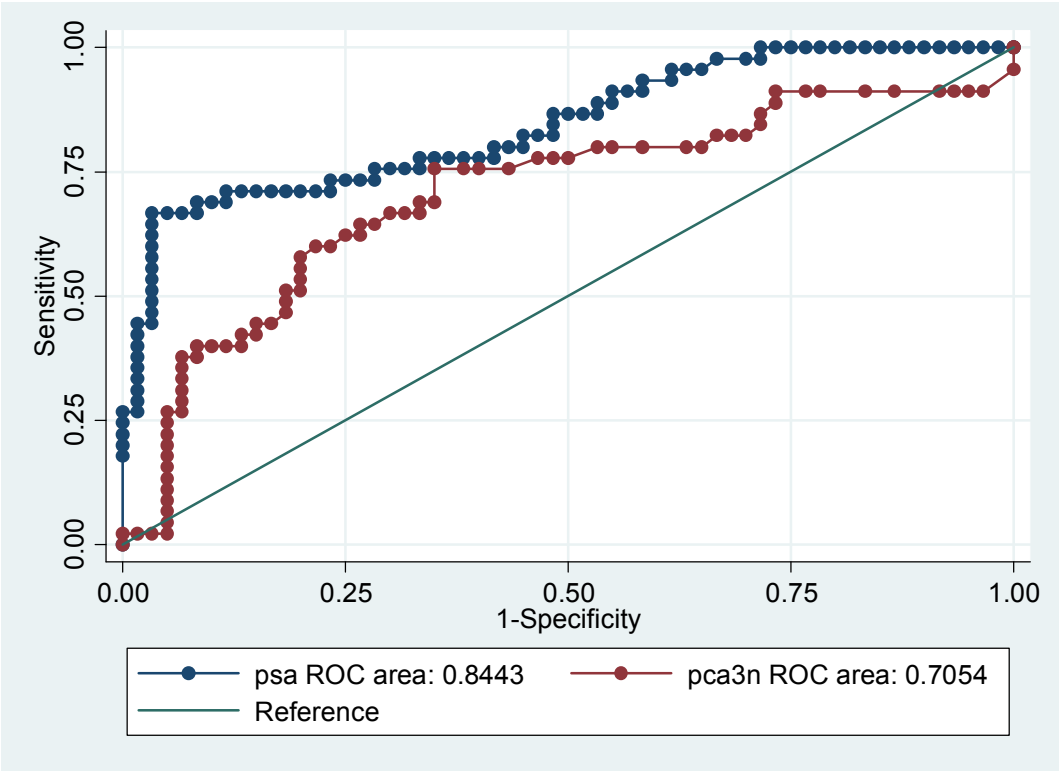
Legends, and the Figures

Figure 1. Bar graphs depicting the percentage of biopsy positive men within the various PCA3 score ranges, with the corresponding patient number in brackets.



Abbreviations: PCA3 = Prostate CAncer gene 3

Figure 2. ROC curve comparison of the PSA level and the PCA3 score.



Abbreviations: ROC = receiver operating characteristics; PSA = prostate-specific antigen; PCA3 = Prostate CAncer gene 3

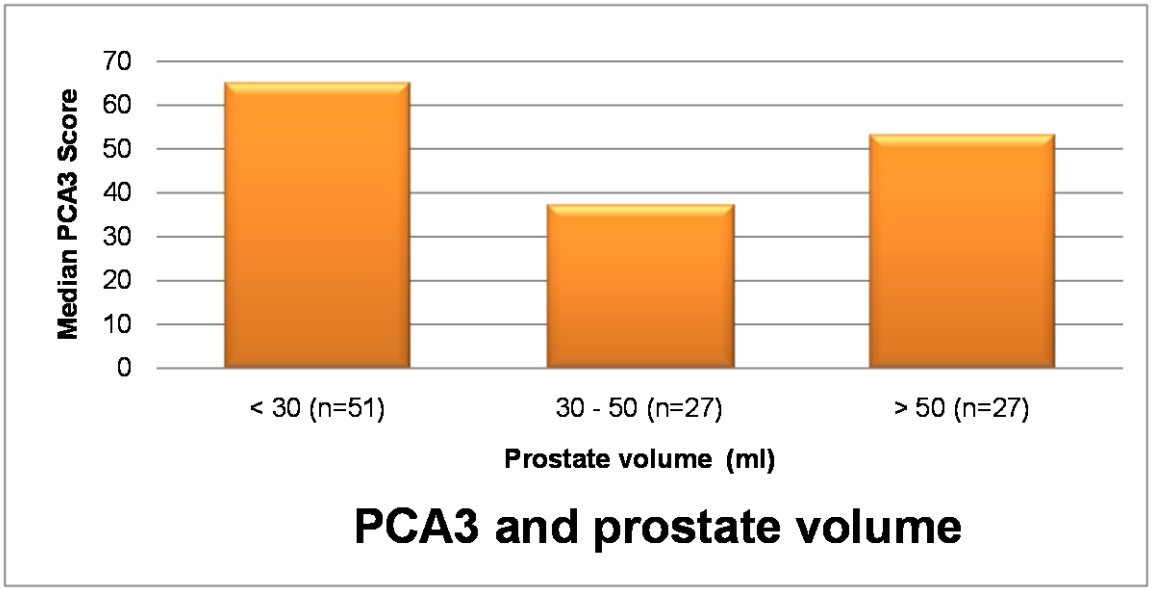


Figure 3a.

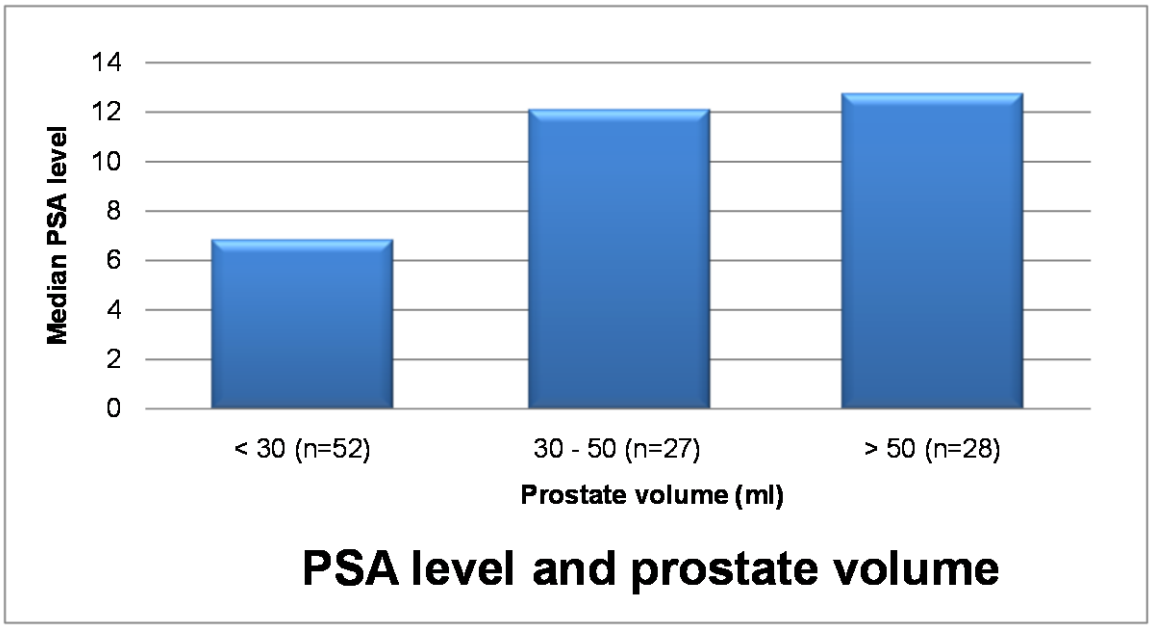
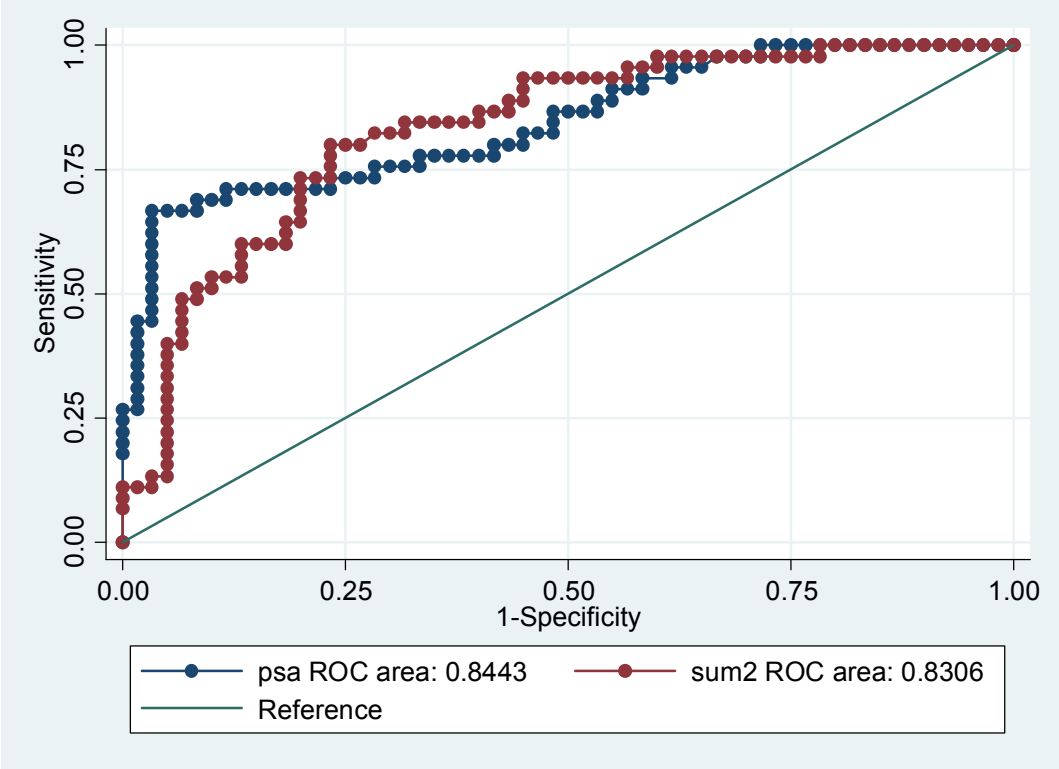


Figure 3b.

Figure 3a & 3b.

Figure 4. The ROC AUC of the sum of PSA and PCA3 against PSA alone.



Abbreviations: ROC = receiver operating characteristics; AUC = area under curve; PSA, prostate-specific antigen; PCA3 = Prostate CAncer gene

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Conflict of interest

None declared.

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