RESEARCH ARTICLE

Comparison of the Formula of PSA, Age, Prostate Volume and Race Versus PSA Density and the Detection of Primary Malignant Circulating Prostate Cells in Predicting a Positive Initial Prostate Biopsy in Chilean Men with Suspicion of Prostate Cancer

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Abstract

Background: Combining risk factors for prostate cancer into a predictive tool may improve the detection of prostate cancer while decreasing the number of benign biopsies. We compare one such tool, age multiplied by prostate volume divided by total serum PSA (PSA-AV) with PSA density and detection of primary malignant circulating prostate cells (CPCs) in a Chilean prostate cancer screening program. The objectives were not only to determine the predictive values of each, but to determine the number of clinically significant cancers that would have been detected or missed. Materials and Methods: A prospective study was conducted of all men undergoing 12 core ultrasound guided prostate biopsy for suspicion of cancer attending the Hospital DIPRECA and Hospital de Carabineros de Chile. Total serum PSA was registered, prostate volumecalculated at the moment of biopsy, and an 8ml blood simple taken immediately before the biopsy procedure. Mononuclear cells were obtained from the blood simple using differential gel centrifugation and CPCs identified using immunocytchemistry with anti-PSA and anti-P504S. Biopsy results were classed as positive or negative for cancer and if positive the Gleason score, number of positive cores and percent infiltration recorded. Results: A total of 664 men participated, of whom 234 (35.2%) had cancer detected. They were older, had higher mean PSA, PSA density and lower PSA-AV. Detection of CPCs had high predictive score, sensitivity, sensibility and positive and negative predictive values, PSA-AV was not significantly different from PSA density in this population. The use of CPC detection avoided more biopsies and missed fewer significant cancers. Conclusions: In this screening population the use of CPC detection predicted the presence of clinically significant prostate cancer better than the other parameters. The high negative predictive value would allow men CPC negative to avoid biopsy but remain in follow up. The formula PSA-AV did not add to the predictive performance using PSA density.

Keywords: Prostate cancer - cancer screening - PSA density - circulating prostate cells

Introduction

Today, a new diagnosis of prostate cancer (PC) nearly always occurs following a patient referral for prostate biopsy (PB) as a result of an increased PSA. PSA is currently the only biomarker used for prostate cancer screening; of men aged 50-70 years 10-20% will have a raised PSA and of men with a PSA of 4-10ng/ml the probability of a positive initial biopsy is approximately 25% (Smith et al., 1997). This probability varies with age, race, family history, PSA level, PSA kinetics, prostate volume and digital rectal examination (DRE). The ability to incorporate these parameters into risk prediction may

decrease the rate of unnecessarily performed biopsies with a decrease in health care costs and side effects. There are an increasing number of predictive tools based on statistical models (Shariat et al., 2008), however many have not been externally validated.

Prostate volume, total serum PSA and age have been found to be clinically significant predictors of positive biopsy findings (Jhiang et al., 2010). Race has also been found to be a predictive factor, afro-americans having a higher risk than white americans. Patel et al. (2012), combined these four predictive factors to produce a simple formula of age multiplied by prostate volume divided by total serum PSA (PSA-AV) then multiplied by a correction

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factor for race. They found that a cut off value of 700 had a sensitivity and specificity of 87% and 35% respectively and performed better than a total serum PSA >4.0ng/ml.

This formula can be re-defined as age divided by prostate density; we present our findings of a Chilean population of men referred from a prostate cancer screening program for a prostate biopsy on the grounds of suspicion of prostate cancer as a result of an elevated serum total PSA. We present a prospective study comparing the formula PSA-AV with that of PSA density (PSA-D) and with the detection of primary malignant circulating prostate cells for the predictive value of detecting prostate cancer at initial biopsy.

The detection of malignant circulating prostate cells (mCPC) could be one candidate for the early detection of PC. In men with prostate cancer there is, at least, one subpopulation of cancer cells that disseminate early, firstly to the neurovascular structures and then into the circulation (Moreno et al., 1992). The number of these cells is very small; however these mCPC can be detected using immuocytochemistry with a combination of anti-P504S (methyl-acyl-CoA racemase) and anti-PSA monoclonal antibodies. The use of the biomarker P504S, although not prostate specific (Zhou et al., 2002), has facilitated the differentiation between normal, dysplastic and malignant tissues in prostate biopsy samples. Normal or benign cells do not express P504S, whereas cells arising from prostatic intraepithelial neoplasia (PIN) or cancer are positive (Beach et al., 2002). The use of primary mCPC detection has been reported to have a high negative predictive value, decrease the number of PB and does not detect low grade small volumen tumors (Murray et al., 2014; Murray et al 2014a).

Materials and Methods

We prospectively studied all men undergoing an initial trans-rectal ultrasound guided (TRUS) prostate biopsy at the Hospital Carabineros of Chile between January 2011 and October 2014. Indications for a TRUS biopsy were an elevated total PSA, defined as >4.0 ng/mL, or a digital rectal examination (DRE) abnormal or suspicious of cancer, defined as the presence of a nodule, areas of indurations, or asymmetry in the size of the lateral lobes (Campbell et al., 2011). The data base created included age and serum PSA and prostate volume. The pathology report of the biopsy was recorded as prostate cancer or no prostate cancer. Blood samples were taken immediately prior to prostate biopsy for the detection of primary circulating prostate cells.

serum total PSA

Total PSA was measured before the digital rectal examination and prostate biopsy (Siemens, AdviaCentaurXR).

TRUS biopsy and prostate volume

PSA density was calculated from the ultrasound findings at the time of biopsy. Trans-rectal ultrasonography of the prostate was performed using an endo-cavitary convex probe with a 6.5MHz transducer (Hitachi,

model EVP-V33). Measures of the tri-axial distances of the prostate were taken in its larger diameter and the total volume was calculated by the following formula: volume=0.52 x transverse diameter x antero-posterior diameter x longitudinal diameter. This volume was used to calculate the PSA density and used in the PSA-AV formula.

All biopsies were standard 12 core, performed transrectally under ultrasound guidance by an experienced urologist using a 18 gauge Tru-Cut needle. Each core was sampled separately, stored in formaldehyde and sent for pathological assessment. A biopsy was defined as positive only when adenocarcinoma as observed in the final histological evaluation. In positive samples the Gleason score, number of positive cores and maximum percent infiltrated was recorded. The pathological analysis and reports were performed by a single dedicated uro-pathologist.

Detection of primary circulating prostate cells

Immediately before the biopsy, an 8mL venous blood sample was taken and collected in a tube containing EDTA (Beckinson-Vacutainer). Samples were maintained at 4ºC and processed within 48 hours. The prostate biopsy and CPC detection were independently evaluated with the evaluators being blinded to the clinical details and results of the biopsy or CPC test.

Collection of CPCs

Mononuclear cells were obtained by differential centrifugation using Histopaque 1,077 (Sigma-Aldrich), washed, and re-suspended in a 100 μL aliquot of autologous plasma. 25 μL aliquots were used to make slides (silanized, DAKO, USA), were dried in air for 24 hours and fixed in a solution of 70% ethanol, 5% formaldehyde, and 25% phosphate buffered saline (PBS) pH 7.4 for five minutes and finally washed three times in PBS pH 7.4.

Immunocytochemistry

mCPCs were detected using a monoclonal antibody directed against PSA, clone 28A4 (Novocastro Laboratory, UK), and identified using an alkaline phosphatase-anti alkaline phosphatase based system (LSAB2, DAKO, USA), with new fuchsin as the chromogen. Positive samples underwent a second process with anti-P504S clone 13H4 (DAKO, USA) and were identified with a peroxidase based system (LSAB2, DAKO, USA) with DAB (3,3 diaminobenzidine tetrahydrochloride) as the chromogen. A mCPC was defined according to the criteria of ISHAGE (International Society of Hemotherapy and Genetic Engineering) (Borgen et al., 1999) and the expression of P504S according to the Consensus of the American Association of Pathologists (Ruben et al., 2001). A mCPC was defined as a cell that expressed PSA and P504S, a benign CPC could express PSA but not P504S, and leucocytes could be P504S positive or negative but did not express PSA. A test was considered positive when at least 1 cell/8mL of blood was detected. P504S was not used alone as leucocytes can be positive for this marker. Patients with benign CPCs were considered as being

negative for the test. Prostate cancer cells as well as PIN cells express P504S whereas benign cells do not; thus cells expressing PSA and P504S were considered to be malignant, whereas cells expressing PSA but not P504S were considered to be benign (Pavlakis et al., 2010).

Analysis of the Results

The discrimination of the three diagnostic tests was defined using the normal parameters: true positive (TP); false positive (FP), false negative (FN), and true negative (TN). The predictive values, positive (PPV)

as well as negative (NPV), were evaluated and the areas under the curve calculated and compared. The potential number of biopsies avoided for each method was calculated and the Gleason scores of missed cancers recorded.

In addition, using the criteria of Epstein (1994), the number of cancers needing active treatment and active observation were registered for each test, whether the test was positive or negative, in order to determine the clinical significance of each test used.

Statistical Analysis

Descriptive statistics were used for demographic variables, expressed as mean and standard deviation in the case of continuous variables with a normal distribution. In case of an asymmetrical distribution the median and interquartile range (IQR) values were used. Noncontiguous variables were presented as frequencies. The Shapiro-Wilk test was used to determine a normal distribution. The Student T-Test was used to compare continuous variables with a normal distribution, the Mann-Whitney test for ordinate and continuous variables with a non-normal distribution, and the Chi-squared test for the differences in

frequency. The diagnostic yield for the test detecting mCPCs and PSA-AV and PSA density score were analyzed using standard parameters. For this purpose patients were classified as having or not having prostate cancer. Statistical significance was defined as a p value less than 0.05; all tests were two-sided. Area under the curve analysis was performed using the online program Vassarcalc.

Ethical Considerations

The study was approved by the hospital ethics committee.

Results

During the study period 664 men, with a mean age of 65.0 +/- 9.1 years, a median total serum PSA of 5.51ng/ml (IQR 4.50-8.00ng/ml) were biopsied. Of these men 234/664 (35.2%) had a biopsy positive for prostate cancer. Men with PC were significantly older 66.4±9.7 years versus 64.3 ± 8.7 years (p<0.006), had a lower median total serum PSA 4.58ng/ml (IQR 4.09-5.39) versus 5.30 (IQR 4.36-7.31 (p<0.001), a higher mean PSA density, 0.16 (IQR 0.11-0.25) versus 0.13 (IQR 0.09-0.17) (p<0.001) and lower PSA-AV, 427 (IQR 271-574) versus 508 (IQR 378-716) (p<0.001). The frequency of circulating tumor cells (CPCs) was significantly higher in the prostate cancer group, 203/234 (87%) versus 57/430 (13% in the no cancer group (p<0.001).

Predictive values

The predictive values of a positive PB for a range of PSA-AV, PSA density and for a range of mCPCs detected/8ml blood simple, and are shown in Tables 1a-c. The predictive values for total serum PSA were not calculated as this was the initial screening test, the three other tests being sequential to a raised total serum PSA. For a PSA-AV cut off point of 700, there was a sensitivity of 85.0 (80.0-89.0), a specificity of 27.0 (23.0-32.0), a PPV of 39.0 (35.0-43.0) and NPV of 77.0 (69.0-83.0).

The areas under the curve were determined for the three tests; for the formula PSA-AV 0.57 (95% CI 0.53-0.61); for PSA density 0.62 (95% CI 0.58-0.66) and for

Table 1. Predicitve Values of PSA-AV, PSA Density and mCPC (95% CI)

		sensitivity	specificity	PPV	NPV
PSA-AV	<400	45.3 (38.8-51.9)	70.0 (65.3-74.3)	45.7 (39.1-52.3)	70.0 (65.0-74.0)
	<800	92.7 (88.4-95.6)	20.5 (16.8-24.7)	38.8 (34.8-52.6)	84.0 (75.1-90.0)
	<1200	98.8 (95.5-99.5)	6.2 (4.3-9.1)	36.3 (26.2-40.2)	87.0 (69.2-95.8)
PSA density	>0.10	85.9 (80.6-90.0)	28.1 (24.0-32.7)	39.4 (35.2-43.8)	78.6 (71.1-84.6)
	>0.15	51.2 (44.7-57.8)	67.7 (63.0-72.0)	46.3 (40.2-52.6)	71.9 (67.2-76.1
	>0.20	31.2 (25.4-37.6)	85.9 (82.1-89.0)	54.5 (45.7-63.0)	69.6 (65.5-73.5)
mCPC	≥1 CPC/sample	87.2 (82.0-91.1)	86.7 (83.1-89.7)	78.2 (72.6-82.9)	92.5 (89.4-94.6)
	≥4CPC/sample	61.1 (54.5-67.3)	94.1 (91.4-96.1)	85.1 (78.6-90.0)	81.6 (77.9-84.9)
	≥8CPC/sample	38.5 (32.3-45.1)	97.2 (95.0-98.5)	88.2 (80.0-93.5)	74.3 (70.5-77.9)

Table 2. Detection of Clinically Significant Prostate Cancer Accordingto Test

	N° Patients	Cancers missed	Needing treatment	Needing observation		N° Patients	Cancers detected	Needing treatment	Needing observation
PSA-AV >700	151	32	19/32 (59%)[a,b]	13/32 (41%)	<700	513	203/513 (40%)	172/203 (85%)	31/203 (15%)[d,e]
PSA density <0.15	372	107	77/107 (72%)[b,c]	30/107 (28%)	>0.15	292	128/292 (44%)	114/128 (89%)	14/128 (11%)[e,f]
mCPC negative	404	32	4/32 (12%)[a,c]	28/32 (88%)	positive	260	203/260 (78%)	187/203 (92%)	16/203 (8%)[d,f]

[a-a] p=0.18; [b-b]p<0.0001; [c-c]p<0.0001; [d-d]p=0.34; [e-e]p=0.02; [f-f]P=0.35

CPC detection 0.86 (95% CI 0.81-0.91). Comparing tests, the formula PSA-AV was not significantly different from the PSA density (p=0.129) whereas primary CPC detection was significantly better than the two PSA derived tests (p<0.0001).

Detection of clinically significant prostate cancer

Of the 234 cancers, 44 (18.8%) complied with the Epstein criteria for active observation (Gleason score ≤ 6; number of positive cores ≤ 2 and ≤50% infiltration in any one core), in other words low grade small volume tumors. There was no significant differences between the ages of men complying with the criteria of active observation versus active treatment; 66.8 +/- 9.9 years versus 66.4 +/- 9.6 years (p=0.96) or median serum PSA 4.88ng/ml (IQR 4.43-6.00) versus 6.24ng/ml (IQR 4.80-10.59) (p=0.19) respectively. For the purpose of the analysis, we defined the following cutoff values to define the need for a prostate biopsy; for the formula PSA-AV a value of less than 700, for PSA density a value >0.15 and for mCPC detection ≥1 cell/8 ml blood detected. For each test we analyzed the number of cancers that would be missed as result of using the specific cutoff value, and the number of cancers that would need treatment or observation according to the Epstein criteria. Similarly we analyzed the number of cancers that would be detected using the defined cutoff values and the number of cancers requiring treatment or observation (Table 2).
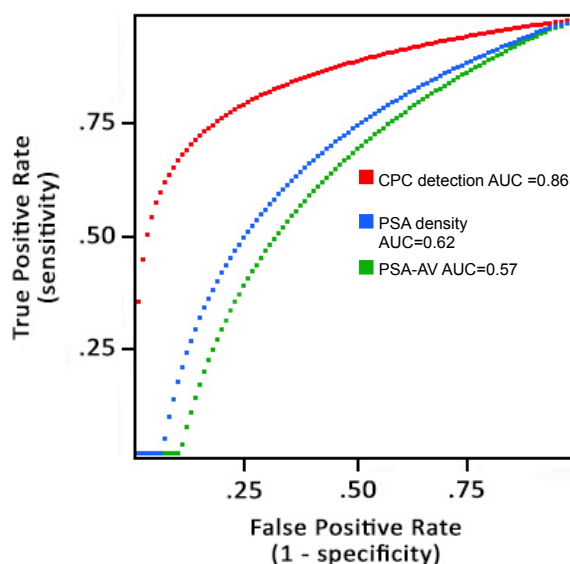
In men with a negative test; there was no difference between PSA-AV and PSA density in the frequency of cancers needing treatment and were not detected. In comparison the number of clinical significant cancers missed using CPC detection was significantly smaller than either of the two PSA based tests, p<0.0001 with the formula PSA-AV and PSA density.

In men with a positive test, there was no difference in the frequency of men detected with a cancer requiring observation, between the formula PSA-AV and PSA density (p=0.34) or between PSA density and CPC detection (p=0.35), less non-significant cancers were detected using CPC detection in comparison with the PSA-AV formula (p=0.02).

The use of primary mCPC detection was clinically superior to both the PSA-AV and PSA density in the detection of cancer. The number of biopsies avoided using the suggested cutoff values, were calculated using the formula: number of patients avoiding biopsy for a negative test-number of biopsies with benign disease detected with a positive test. The values for the formula PSA-AV were 151-(513-310) =-52/664 (-8%); for PSA density 372-(292-128)=208/664 (31%) and for mCPC detection 404-(260-203)=347/664 (52%).

The number of biopsies avoided was significantly higher using mCPC detection; mCPC versus PSA density, 347 versus 208 p<0.0001; both mCPC detection and PSA density were superior than PSA-AV (p<0.0001). The formula PSA-AV did not produce a net saving of biopsies, more men with benign disease underwent biopsy using a cutoff value of 700 than men avoiding biopsies.

The cost of these avoided biopsies was the number of prostate cancers missed, especially those cancers clinically

significant. Of the men who would not have been biopsied; the formula PSA-AV would have missed 19/151 (13%) clinically significant cancers; PSA density 77/372 (21%) and mCPC detection 4/404 (1%) respectively. PSA density missed significant more important cancers than the other two detection methods; versus PSA-AV (p=0.03); versus mCPC (p<0.0001), PSA-AV missed more significant cancers than mCPC detection (p<0.0001).

There were no significant differences in the frequency of clinically significant cancers detected in men with positive tests.

Discussion

The limitations in the sensitivity and specificity of total serum PSA values remain problematic (Welch et al; 2005). One approach to predict the likelihood of a positive biopsy is to combine risk factors within a nomogram. The PSA-AV formula is one such nomogram which has been externally validated (Patel et al., 2012). In our study the PPVs at given PSA-AV cutoff values were lower than that reported by Patel et al. (2012), this may be a race factor, the Chilean population is of diverse ethnic origin and as such does not fit into distinct ethnic origins, such as Caucasian, Afro-American, Hispanic. The PSA-AV formula was not superior in predicting a positive biopsy when to compare to PSA alone or PSA density. Our results using PSA density differed from those reported by Lodeta et al. (2009), which suggests that differing populations need to have their standard cutoff values. One drawback that affects both PSA density and the formula is the need for prostate volume estimation. Prostatic ultrasound is not routine in Chile, the prostate volume being calculated at the time of biopsy and so does not influence the clinical decision as whether to carry out a biopsy or not.

The use of mCPC detection does not require prostate volume estimation and proved in our series to be superior to the PSA-AV and PSA density in predicting the presence of clinically significant prostate cancer. An ideal biomarker should only detect disease that will probably affect the survival or quality of life; it has been estimated that 23-42% of screen detected prostate cancers are over-treated

(Draisma et al., 2009). If active surveillance is considered the best option for patients with low risk cancers or a short life expectancy, then the aim of the prostate biopsy in men with an elevated PSA is not to detect each and every prostate cancer but to detect those prostate cancers with the potential for causing harm during the patient´s lifetime. Men with clinically insignificant prostate cancers that were never destined to have symptoms or to affect their life expectancy may not benefit from knowing that they have the "disease." The detection of clinically insignificant prostate cancer could be considered as an adverse effect of the prostate biopsy. As such, there is considerable anxiety and distress found in men undergoing active surveillance (van de Bergh, 2009).

The use of primary mCPC detection as a sequential test in men with suspicion of prostate cancer due to an elevated total serum PSA was superior to both the Montreal score and free percent PSA, both as a predictive test and more importantly the number of clinically significant cancers that would be missed in mCPC negative men. It is important to note that the use of mCPC detection is designed as a sequential test, for men with an abnormal PSA or DRE, that mCPC positive cases should be evaluated with prostate biopsy and mCPC negative cases followed up. That the test is positive or negative with no cut-off point simplifies clinical decisions as to whether proceed to prostate biopsy. This is reinforced by the high negative predictive value of the test, 94% of mCPC negative men did not have cancer detected on the initial biopsy, and the fact that the 6% of men with cancer had low grade small volume tumors. That low grade small volume cancers were mCPC negative fulfills the concept that not all tumors need to be detected.

An ideal biomarker for the detection of prostate cancer is one that detects clinically significant cancers, does not detect indolent cancer, and has a high negative predictive value to avoid unnecessary biopsies. Prostate biopsies have associated risks; with a 30 day complication rate of 3.7%, especially in those over 85 years or with three or more comorbidities and has increased in recent years predominantly as a result of infection (Anastasiadis et al., 2014) and thus avoiding biopsies is a worthwhile aim, if the number of clinically significant cancers detected is not prejudiced.

Studies detecting circulating prostate cells, using different methodologies have been discordant results. They have been reported in between 37-80% of studies using EpCAM (Epithelial Cell Adhesion Molecule) based detection systems (Fizazi et al., 2007; Davis et al., 2008; Eschwege et al., 2009; Stott et al., 2010). The failure to include tumor cells that have reduced or absent EpCAM expression may limit investigations and fails to differentiate between benign and malignant circulating prostate cells. EpCam is expressed in most but not all tumors (Went et al., 2004) and there is down regulation with cancer progression and metastasis. In addition during epithelial to mesenchymal transition the expression of EpCAM is down regulated (Paterlini-Brechot, 2007), allowing epithelial cell dissociation and dissemination from the primary tumor (Raimondi et al., 2011). In this study the use of PSA and P504S to define CPCs avoids

this problem, and the results are similar to that of Fizazi et al. (2007) who also avoided the use of an EpCAM based system.

The failure to distinguish between benign and malignant CPCs may explain in part the failure of EpCAM based systems to differentiate between cancer and control patients, benign CPCs do not express P504S (Murray et al., 2013). This underlies the problem with the different methods used to detect circulating tumor cells and has been extensively reviewed (Panteleakou, 2009).

Our results suggest that the use of primary mCPCs is superior to the use of the formula PSA-AV and PSA density in predicting the results of the initial prostate biopsy in men with suspicion of prostate cancer as a result of an elevated PSA and/or abnormal DRE. It does not detect low grade small volume tumors and its high negative predictive value suggests that mCPC negative patients need not undergo the risks of prostate biopsy. What the results suggest is that the mCPC test is superior to the other predictive methods, not in the prediction of finding a clinically significant prostate cancer, but predicting when there is not a clinically significant prostate cancer. This is fundamental to avoid unnecessary biopsies, in men with a total serum PSA of 4.0-10.0ng/ml, 70% will have a biopsy negative for cancer, a predictive model should be able to identify these patients without missing clinically significant cancers. Although the number of biopsies avoided using mCPC detection is lower than the other models, the number of clinically significant cancers is much less, and in terms of clinical practice is important. Thus as a predictive test to exclude men the use of mCPCs is clinically significant.

In addition, a prostatic ultrasound is not required in the decision making process in contrast to PSA-AV and PSA density. Although Patel et al. (2014) suggest that the formula has use in the elderly with large prostates, the use of mCPC proved to be superior than age related PSA in a fit Chilean elderly population (Murray et al., 2015).

The detection of mCPC is a simple test which could be incorporated in the routine immunocytochemical laboratory of a general hospital. The test can be semi-automated; there are commercial systems that are automatized for immunocytochemistry, that permit double immune-marcation of cells. In terms of costs, it has been shown to be cost effective when used as a sequential test for the number of biopsies avoided (Murray et al., 2013a), which is important for a public health system.

We recognize that our study has various limitations; firstly it is a single center study, where the immunocytologist has the experience and training to perform the tests and has been internally validated as to pre-analytical, analytical and post analytical variables as described in the methods section. That this study is focused on patients with suspicion of prostate cancer (abnormal PSA and/or DRE) and may not reflect the general prostate cancer screening population. However, previously published work has reported that there is an association between total serum PSA and mCPC detection frequency, ranging from 5% in patients with a total serum PSA of <2.0ng/ml to 42% in patients with a PSA >10.0ng/ml (30). However, we consider that the study population represents "real life"

Nigel P Murray et al

practice where the patient has been referred from primary care services for consideration of a prostate biopsy. It is the decision of the urologist whether to proceed to biopsy or not. Both the PSA-AV formula and PSA density have a threshold probability above and below which the urologist has to recommend or not a biopsy. Currently a definitive acceptable threshold does not exist. The mCPC test is avoids this problem by being a positive/negative test and thus is easier to use.

In conclusions, the use of the formula PSA-AV in Chilean men did not prove superior to the use of PSA density alone. The use of primary mCPC detection was superior in the prediction of initial prostate biopsy results than both the formula PSA-AV and PSA density; it was also superior in that the number of missed clinically significant cancers yielded by a negative test was significantly lower. warrants multicenter studies to confirm these results.

Acknowledgements

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100.0

6.3

75.0

56.3

50.0

25.0

31.3

0

Newly diagnosed without treatment