RESEARCH ARTICLE

Head to Head Comparison of the Chun Nomogram, Percentage Free PSA and Primary Circulating Prostate Cells to Predict the Presence of Prostate Cancer at Repeat Biopsy

Nigel P Murray[1,2]*, Eduardo Reyes[1,3], Nelson Orellana[1], Cynthia Fuentealba[1], Omar Jacob[1]

Abstract

Background: The limitations of total serum PSA values remain problematic, especially after an initial negative prostate biopsy. In this prospective study of Chilean men with a continued suspicion of prostate cancer due to a persistently elevated total serum PSA, abnormal digital rectal examination and initial negative prostate biopsy were compared with the use of the on-line Chun nomagram, detection of primary malignant circulating prostate cells (CPCs) and free percent PSA to predict a positive second prostate biopsy. We hypothesized that men negative for circulating prostate cells have a small risk of clinically significant prostate cancer and thus may be conservatively observed. Men positive for circulating prostate cells should undergo biopsy to confirm prostate cancer. Materials and Methods: Consecutive men with a continued suspicion of prostate cancer underwent 12 core TRUS prostate biopsy; age, total serum PSA and percentage free PSA and Chun nomagram scores were registered. Immediately before biopsy an 8ml blood simple was taken to detect primary mCPCs. Mononuclear cells were obtained by differential gel centrifugation and identified using double immunostaining with anti-PSA and anti-P504S. Biopsies were classifed as cancer/no-cancer, mCPC detecton test as negative/positive and the total number of cells/8ml registered. Areas under the curve (AUC) for percentage free PSA, Chun score and CPCs were calculated and compared. Diagnostic yields were calculated with reference to the number of possible biopsies that could be avoided and the number of clinically significant cancers that would be missed. Results: A total of 164 men underwent a second biopsy; 41 (25%) had cancer; the AUCs were 0.65 for free PSA, 0.76 for the Chun score and 0.87 for CPC detection, the last having a significantly superior prediction value (p=0.01). Using cut off values of free PSA <10%, Chun score >50% and ≥1 CPC detected, CPC detection had a higher diagnostic yield. Some 4/41 cancers complied with the criteria for active surveillance, free PSA and the Chun score missed a higher number of significant cancers when compared with CPC detection. Conclusions: Primary CPC detection outperformed the use of free PSA and the Chun nomagram in predicting clinically significant prostate cancer at repeat prostate biopsy.

Keywords: Prostate cancer - circulating prostate cells - repeat prostate biopsy - Chun score - free PSA - predictive value

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Introduction

Approximately 70% of men with a raised total serum PSA, defined as >4.0ng/ml will have an initial prostate biopsy negative for cancer. However, a persistently elevated PSA after initial biopsy is a relevant clinical problem and the decision to repeat the prostate biopsy in these patients is a common dilema. Prostate biopsy is not without morbidity; 1% of patients suffer sepsis or severe hemorrhage and 5% urinary tract infections (Rodriguez et al, 1998; Anastasiadis et al., 2014). The need to reduce unnecessary biopsies has been highlighted in national guidelines (Graham et al., 2008). Twenty percent of prostate cancers are detected at the second prostate biopsy (Djavan et al., 2000) and a number of parameters have been recommended to reduce the frequency of a benign repeat biopsy. Abnormal digital rectal examination (DRE), free to total PSA ratio, a PSA velocity >0.75ng/ml/year or a PSA density >0.15 (Djavan et al., 2000; Fowler et al., 2000; Eggener et al., 2005) have all been propoed but as individual parameters there have poor positive and negative predictive values (Djavan et al., 2000). There are an increasing number of predictive tools based on statistical models (Shariat et al., 2008) to predict a positive repeat biopsy, however many have not been externally validated. The Chun´s nomogram predictive tool has been externally validated (Karakiewicz et al., 2005), it uses simple readily available markers, age, DRE, PSA and

*[1]Hospital Carabineros of Chile, Nunoa, [2]Faculty of Medicine, University Finis Terrae, Providencia, [3]Faculty of Medicine, Diego Portales University, Manuel Rodrıguez Sur, Santiago, Chile *For correspondence: nigelpetermurray@gmail.com*

percent free PSA and prostate volume to give a percent risk calculation in an individual patient.

The objective of this study was to compare the Chun´s nomogram and free percent PSA with the detection of primary circulating prostate cells to predict the results of a second prostate biopsy in men with a persistently elevated or rising serum PSA and/or abnormal DRE. The detection of malignant circulating prostate cells (CPC) 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 CPC 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 CPC 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 transrectal ultrasound guided (TRUS) prostate biopsy at the Hospital Carabineros of Chile between January 2006 and December 2010. 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 free percentage PSA. The pathology report of the biopsy was recorded as prostate cancer or no prostate cancer. Blood samples were taken immediately prior to the initial prostate biopsy for the detection of primary circulating prostate cells. Men diagnosed as having prostate cancer were treated according to national guidelines. Men without prostate cancer were followed up until December 2014, the treating urologist recommended a second biopsy if the DRE was considered abnormal; a serum total PSA >4ng/ml or a PSA velocity >0.75ng/ml/year. Repeat blood samples were taken immediately prior to the second prostate biopsy for the detection of primary circulating prostate cells.

a). TRUS biopsy

All biopsies were standard 12 core, performed transrectally under ultrasound guidance by an experienced urologist using a 18 guage 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 deciated uropathologist.

b) Chun's nomogram

Risk assessment of a positive biopsy outcome was performed using the online based risk calculator for the Chun model (http://nomogram.org/prostate/pros_calc.php). Total PSA and % free PSA were measured before the DRE using the automatic system Siemens,AdviaCentaurXR system for total PSA and % free PSA.

Transrectal ultrasonography of the prostate was performed using an endocavitary convex probe with a 6.5MHz transducer (Hitachi, model EVP- V33). Measures of the triaxial distances of the prostate were taken in its larger diameter and the total volume was calculated by the following formula:

volumen = 0.52 x transverse diameter x anteroposterior diameter x longitudinal diameter. This volume was used in the Chun´s nomogram.

c) 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.

i) Collection of CPCs: Mononuclear cells were obtained by differential centrifugation using Histopaque 1,077 (Sigma-Aldrich), washed, and resuspended in an 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 mintues and finally washed three times in PBS pH 7.4 .

ii) 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 (Rubin 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).

Slides were analyzed manually, stained cells were photographed using a digital camara and from the digital images determined if mCPCs were present or absent and the total number of mCPCs detected by one trained observer. Using this method, in preliminary workup trials of 50 patients using three observers, there was agreement in 86% of cases between the three observers, in 14% of cases between two observers. The differences in opinion were on scoring P504S between +1 and +2 scores which affected the total mCPC count but not if the test was positive or negative. As the test is designed as a positive/negative test it was considered appropiate to proceed, aknowledging that there is an interobserver variation in the absolute mCPC count.

Analysis of the Results

The discrimination of the two 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 et al. (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 nonnormal distribution, and the Chi-squared test for the differences in frequency. The diagnostic yield for the test detecting mCPCs and Chun´s 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 value less than 0.05, all tests were two-sided. Area under the curve analysis was performed using the online programe Vassarcalc.

Ethical Considerations

The present study was approved by the hospital ethics committee.

Results

A total of 559 men underwent an initial prostate biopsy, of whom 183 (32.7%) were diagnosed as having cancer. The 376 men with a benign pathology were followed up, of whom 164/376 (43.6%) underwent a second biopsy.

These men had a mean age of 65.1 ± 8.5 years, a median total serum PSA of 6.18ng/ml (IQR 4.95-9.26ng/ml), a median free percent PSA of 15% (IQR 11-19%) and a median prostate volume of 56ml (IQR 42-67ml). Of the 164 second biopsies, 41/164 (25%) were positive for cancer.

a) Discriminative power: areas under the curve were calculated for primary CPC detection, free percent PSA and Chun´s nomogram; giving the following respective results, 0.873 for CPC detection, 0.648 for percent free PSA and 0.755 for Chun´s nomogram. The discriminative power of CPC detection was significantly superior to free percent PSA ($p<0.0004$) and the Chun´s nomogram ($p<0.05$), there was no significant difference between the free percent PSA and Chun´s nomogram ($p=0.13$). (Figure 1).

b) Predictive values: The predictive values of a positive biopsy for a positive/negative CPC test, and for a range of free percent PSA values and Chun´s nomogram scores are shown in Tables 1-4. The presence of CPCs was associated with a positive biopsy ($p<0.0001$) with a relative risk of 36.60 (95% CI 9.17-146) and an odds ratio of 113.8 (95% CI 25.2-513). Using a free percent PSA cut off value of <10% and < 15% the predictive values are shown in Table 3.

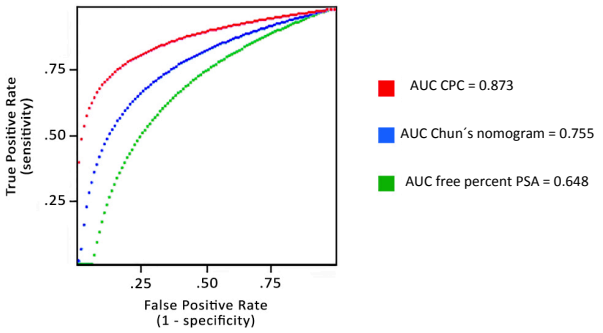

Figure 1. Area under the curves for CPC detection; Chun`s nomograma and free percent PSA

Table 1. Results of CPC Detection and Second Prostate Biopsy

	Biopsy cancer	Biopsy no cancer	Total
CPC positive	39	18	57
CPC negative	2	105	107
Total	41	123	163

Table 2. Predicitve Values of CPC Detection

	value	95% CI
sensitivity	95%	82-99%
specificity	85%	82-99%
positive predictive value	68%	55-80%
negative proedictive value	98%	93-99%
(+) likelihood ratio	6.5	4.2-10.0
(-) likelihood ratio	0.06	0.02-0.22

Table 3. Predicitve Values of Free Percent PSA with Cutoff Values of <10% and < 15%

	≤ 10% value	95% CI	≤ 15% value	95% CI
sensitivity	34%	21-51%	83%	67-92%
specificity	83%	75-89%	49%	40-58%
positive predictive value	40%	24-58%	35%	26-46%
negative predicitve value	60%	42-76%	90%	79-95%
(+) liklihood ratio	2.0	1.12-.56	1.62	1.30-2.02
(-) liklihood ratio	0.79	0.68-0.99	0.35	0.18-0.70

Table 4. Predicitve Values of Chun´s Nomograma with Cutoff Values of >25% and > 50%

	>25% value	95% CI	>50% value	95% CI
sensitivity	88%	82-93%	61%	45-75%
specificity	12%	7-18%	74%	65-81%
positive predictive value	28%	21-36%	44%	31-58%
negative predicitve value	95%	72-99%	85%	77-91%
(+) liklihood ratio	1.14	1.05-1.25	2.34	1.59-3.45
(-) liklihood ratio	0.17	0.02-1.27	0.53	0.37-0.78

Table 5. Detection of Clinically Significant Prostate Cancer According to Test

	Nº patients	cancers missed	needing treatment	needing observation		Nº patients	cancer detected	needing treatment	needing observation
free PSA > 10%	128	27	24/27 (89%)	3/27 (11%)	free PSA < 10%	36	14	13/14 (93%)	1/14 (7%)
Chuns nomogram <50%	107	15	14/15 (87%)	1/15 (1%)	Chuns nomogram > 50%	57	26	23/26 (89%)	3/26 (11%)
CPC negative	107	2	0/2 (0%)	2/2 (100%)	CPC positive	57	39	37/39 (95%)	2/39 (5%)

Using a Chun´s nomograma cutoffvalues of >25% and >50%, the predictive values are shown in Table 4.

CPC subgroup analysis

Of the men undergoing a second biopsy, 17/164 (10%) were CPC (+) prior to the first biopsy. Of these 17 men, 14/17 (82%) had a cancer detected at the second biopsy, 2 men remained CPC positive with a negative biopsy and 1 man changed from positive to negative with a negaive biopsy.

147/164 (90%) were CPC negative prior to the first biopsy, of these 41 were CPC positive prior to the second biopsy, of whom 25/41 (61%) had a second biopsy positive for cancer, of the 106 who had a second negative CPC test, two men had a positive biopsy for cancer, both Gleason 4 tumors.

Detection of clinically significant prostate cancer

Of the 41 cancers, 4 cancers complied with the Epstein criteria for active surveillance, (Gleason score ≤ 6; number of positive cores ≤ 2 and ≤50% infiltration in any one core) in other words low grade small volumen cancers. For the purpose of the analysis, we defined the following cutoff values to define the need for a prostate biopsy; for free percent PSA a value of less than 10%, for the Chun score a probability of >50% and for CPC 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 accodring to the Epstein criterio. 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).

The three tests when positive, detected clinically significant prostate cancers with the same frequency (between 89-95%); however the frequency of significant cancers detected with respect to the number of patients biopsied was significantly higher using CPC detection in comparison with free percent PSA (p<0.01 OR 3.40 95% CI 1.42-8.14); CPC detection versus Chun´s nomogram (p<0.02 OR 2.58 95% CI 1.20-5.55) and no significant difference between free percent PSA and Chun´s nomogram (OR 1.32 95% CI 0.56-3.08).

Inversely, when the test was considered negative, the free percent PSA missed significantly more cancers than CPC detection (p<0.001 OR 14.04 95% CI 3.25-60.56) as did the Chun´s nomogram (p=0.003 OR 8.56 95% CI 1.91-38.43). There was no significant difference between the free percent PSA and the Chun´s nomogram. The missed cancers were clinically significant, in otherwords needed to be treated in both the free percent PSA and Chun´s nomogram negative groups.

Discussion

Urologists use various parameters when deciding to repeat a prostate biopsy, DRE findings, serum total PSA, free percent PSA, PSA velocity have all been used in this setting. The predictive value of these individual parameters is limited leading to refine the predictive process and reduce the number of unnecessary repeat biopsies. To this end nomograms and artificial neural networks have been used but have not gained widened acceptance (Carlson et al., 1998; Djavan et al., 2002;

Lopez et al., 2003).

Identifying men who require a repeat biopsy whilst avoiding unnecessary biopsies is not straightforward, the free percent PSA has been reported to have an AUC of around 74% (Djavan et al., 2002), in our small group free percent PSA had an AUC of 64%, the Kattan nomogram using a combination of parameters attained an AUC of 71% (Yanke et al., 2005), that of the Chun nomogram 76% (Chun et al., 2007), which was similar to that obtained in our study group.

The use of primary CPC detetction as a sequential test in men with suspicion of prostate cancer and a negative initial biopsy was superior to both the Chun score and free percent PSA, both as a predictive test and more importantly the number of clinically significant cancers that would be missed in CPC negative men.

That the test is positive or negative with no cut-off point simplifies clinical decisions as to whether proceed to repeat prostate biopsy. This is reinforced by the high negative predictive value of the test, 98% of CPC negative men did not have cancer detected at the second biopsy, and the fact that the 2% of men with cancer had low grade small volume tumors. That low grade small volumen cancers were CPC negative fulfills the concept that not all tumors need to be detected. Thus men with a persistently elevated total serum PSA and CPC negative could continue to be observed rather than undego biopsy.

Studies using different methods to detect CPCs have reported discordant results, 37-80% of cases using an EpCAM (epitelial cell adhesion molecule) based detection systems (Fizazai et al., 2007; Davis et al., 2008; Eschwege et al., 2009). 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) with downregulation with cancer progression and metastasis. Also EpCAM is downregulated during epithelial to mesenchymal transition (Paterlini et al., 2007), permitting 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 et al., 2009).

Using the PSA-P504S combination of biomarkers our results suggest that the use of primary CPCs is superior to the use of free percent PSA and the Chun score in predicting the results a repeat prostate biopsy in men with continued suspicion of prostate cancer. It does not detect low grade small volumen tumors and its high negative predictive value suggests that CPC negative patients need not undergo the risks of prostate biopsy.

It 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 comercial systems that are automatized for immunocytochemistry, that permit double immunomarcation 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.

Our study has various limitations, firstly it is a single centre study, where the immunocytologist has the experience and training to perform the tests and has been internally validated as to pre-analytical, analylitical and post analytical variables as described in the methods section. This study is focussed on patients with suspicion of prostate cancer (abnormal PSA and/or DRE) and may not reflect the general prostate cancer screening population. However, we consider that the study population represents "real life" practice, with the uncertainities of whether or not a biopsy should be repeated. Both the Chun nomogram and free percent PSA do not have a definitive threshold to recommend repeat biopsy; whereas the CPC test gives a positive/negative result thus facilitating management decisions.

Conclusions: The use of primary CPC detection was superior in the prediction of a repeat prostate biopsy results than both the Chun nomogram and the free percent PSA, it was also superior in that the number of missed clinically significant cancers yielded by a negative test was significantly lower. It warrents multicentre studies to confirm these results.

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