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# Future Aspects of Prostate Biopsy – The Use of Primary Circulating Prostate Cells to Select Patients for Prostate Biopsy: Evidence, Utility and Cost-Benefit

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## 1. Introduction

Serum prostate specific antigen (PSA) is the only biomarker routinely used for the early detection of prostate cancer, but it is not a perfect test. Although PSA is highly specific for prostate, an elevated level is not specific for cancer, being increased in benign hyperplasia and prostatitis (Pungalia, 2006; Bozeman, 2002). Consequently, the majority of men with an increased serum PSA do not have prostate cancer and thus undergo unnecessary prostate biopsies.

Data from the USA estimate that of the million prostate biopsies performed annually, only 235,000 cases of cancer are detected, or that more than 750,000 men underwent a biopsy based on an elevated PSA caused by benign disease (Fadore, 2004; Jemal, 2006). Published data from the Prostate Cancer Prevention Trail showed that there is no cut-off point for serum PSA; for values up to 4ng/ml the sensibility of the test showed a variation of between 21% and 83%, a specificity of between 39% to 94%, with a positive predictive value of between 7% and 27% (Thompson, 2005).

## 2. Current indications for a prostate biopsy and the use of serum PSA

### 2.1 Controversies about what level of serum PSA should indicate a biopsy

At present the indications for a prostate biopsy are an abnormal digital rectal examination (DRE) and /or an increased serum PSA. However, the sensibility and specificity varies with race and the cut-off point used to indicate a biopsy. In the Finnish population, using a cut-off point of 3ng/ml and 4ng/ml the sensibility was 89% and 87% respectively (Auvienen, 2004), in Russia using a cutoff point of 4ng/ml the sensibility and specificity were 92% and 63% respectively (Matveev, 2006), while in the United States the values were 90% and 73% respectively (Labrie, 1992). Although a PSA level of 4 ng/ml is used as a cut-off point, 22%

of men with a PSA level of between 2.5 and 4.0 ng/ml have been shown to have clinically significant organ confined prostate cancer (Catalona, 1997; Horninger, 2004; Thompson, 2004). Or in other words, 62% of men with prostate cancer have a serum PSA >4.0ng/ml y 70% of all men with a serum PSA >4.0ng/ml do not have prostate cancer.

### 2.2 False positive rate of serum PSA and implications: Costs, increased follow-up, collateral effects of unnecessary prostate biopsies (including direct, sepsis, hemorrhage y indirect anxiety, increased follow-up)

Ideally a screening test should detect all clinically significant prostate cancers and not benign pathologies. It has been normal practice that men who are found to have an abnormal serum PSA level should have a prostate biopsy. For example, the UK Prostate Cancer Risk Management Programme (PCRMP) states "if your PSA is definitely raised, a prostate biopsy is required to determine whether cancer is present" The justification for performing biopsy in men with an abnormal PSA is that they are at high risk of prostate cancer. However, data from the Prostate Cancer Prevention Trial (Thompson, 2006) and Baltimore Longitudinal Study on Aging (Fang, 2001) have demonstrated that prostate cancer is also a common finding on biopsy in men with a *normal* PSA level. The data from this large study provide a strong argument against the use of an arbitrary PSA threshold to select men for prostate biopsy. The aim of prostate biopsy is not to detect each and every prostate cancer. After all, the Prostate Cancer Prevention Trial demonstrates that the majority of prostate cancers are in men with a normal PSA level. The aim of prostate biopsy is actually to detect those prostate cancers with the potential for causing harm.

It has been estimated that, of asymptomatic men in whom prostate cancer is detected by prostate biopsy following PSA measurement, around 50% (Draisma, 2003) do not require active treatment. Men with clinically insignificant prostate cancers that were destined never to cause any symptoms, or affect their life expectancy, may not benefit from knowing that they have the 'disease'. Indeed, the detection of clinically insignificant prostate cancer should be regarded as an (under-recognised) adverse effect of biopsy. In order to identify men who are most suitable for prostate biopsy, there is a need to identify a group at high risk, not just of prostate cancer, but of *significant* prostate cancer. Several large studies have analyzed the clinical characteristics associated with the finding of higher grade (usually defined as Gleason score ≥7) prostate cancer on biopsy. Factors significantly associated with high grade cancer were: PSA level, smaller prostate volume, abnormal DRE findings, age, and black African and black Caribbean ethnicity, whereas a previous negative prostate biopsy reduced this risk.

A false-positive PSA (or a PSA >4.0ng/mL) has consequences, firstly the collateral effects or complications of a prostate biopsy, the additional follow-up and possibility of a second or third biopsy. Observational studies, and theoretical considerations, suggest that re-biopsy will detect prostate cancer in some men with an initially negative prostate biopsy. These studies reported multivariate analyses of predictive factors for positive repeat biopsy but there was disagreement on which factors predict re-biopsy outcome. There is evidence, however, that the odds of high grade prostate cancer are reduced if a man has previously had a negative biopsy.(Djavan, 2000; Eggener, 2005; López-Corona, 2003; Mian, 2002; Roobal, 2006)

Using the results from the European Prostate Cancer Detection Study, there were no significant differences found in the tumor characteristics of stage and Gleason score comparing the first and second biopsy. The results have shown that with a second biopsy,

prostate cancer is detected in between 10% and 31% of cases (Yuen, 2004; López-Corona, 2006). However, there are patients with two negative biopsies who continue with a high suspicion of cancer, with a persistently elevated PSA or pre-malignant histology report such as prostate intraepithelial neoplasia grade 3 or atypical microacinar proliferation. The evidence suggests that cancers diagnosed with the third or fourth biopsy are those of low grade and volume (Djavan, 2001). The key question is how often is it justified to re-biopsy the patient that perhaps does not have a cancer that is life threatening. The diagnosis of a prostate cancer not clinically significant implies an overdiagnosis and over-treatment. However, there are no predictive factors, clinical or laboratorial that help to differentiate patients between men with clinically significant or not significant prostate cancer. At present the only factor available is the result of the prostate biopsy.

A prostate biopsy is not without its complications; Rietbergen et al (1993) in a study of 1687 patients reported an incidence of hematuria, hematospermia and fever in 23.6%, 45.3% and 4.2% of patients respectively. More severe complications requiring hospitalization occurred in 0.4% of patients. Moreover, Gallina et al (2008) analyzed the mortality at 120 days after an ultrasound guided prostate biopsy, the study realized in the years 1989-2000 included 22,175 patients and 1,778 controls, the mortality reported was 1.3% in biopsied men versus 0.3% in the control group.

### 2.3 Other indicators available based on serum PSA

Thus, a search for new biomarkers which could be more specific for the detection of prostate cancer is needed. The use of biomarkers such as percent free PSA (Lee, 2006), intact serum PSA (Steuber, 2002), serum pro-PSA(Lein, 2005) and kallikrein (Stephan, 2000) have shown to be useful in the detection of prostate cancer. However, although a biomarker could improve the precision of screening it is possible that in clinical practice it is not viable, for the need of fresh samples or high costs (Villanueva, 2006).

The use of PSA velocity has been suggested, an increase of more than 0.75ng/ml/year has been associated with an increased risk of prostate cancer and increased specific mortality (Carter, 1992; D´Amico, 2004; Heindenreich,2008). However, more recent studies have put in doubt the true role of PSA velocity, the European Randomized Study of Screening for Prostate Cancer demonstrated that increased PSA velocity was not associated with increased cancer risk, but was associated with higher grade cancers, defined as ≥ stage T1c and/or a Gleason score ≥7 (Roobol, 2006a).

Age and race adjusted PSA values has also been called in question, evaluating whether or not the PSA age adjusted range is sufficient to eliminate the need for a biopsy, revealed that 54% of patients who would not be biopsied using these criteria, had a high grade cancer diagnosed (Wolff, 2000). Similarly the free-PSA fraction has been called into doubt for the same cut-off value as with total serum PSA.

## 3. Circulating prostate cell detection

### 3.1 Theory of primary CPCs and experimental evidence

One possible candidate is the detection of circulating prostate cells (CPCs). In men with prostate cancer there is, at least, one subpopulation of cancer cells that disseminate early, to the neurovascular structures and then to the circulation (Moreno, 1992). The number of cells is very small and not detected by conventional tests; however these CPCs can be detected using immunocytochemistry.

PSA is not specific for prostate cancer, circulating prostate cells have been detected in cases of prostatitis.(Murray, 2010) thus PSA expressing cells detected in blood may not represent malignancy, but benign cells that have escaped into the blood due to acute inflammation of the prostate gland. P504S (methylacyl-CoA racemase) is an enzyme that is expressed in dysplastic and malignant prostate tissue but not by normal prostate cells.(Rubin, 2002; Luo,2002) . As dysplastic cells do not disseminate, those prostate cells expressing P504S in the circulation are considered to be malignant. However, P504S is not specific to the prostate; it is expressed in normal and malignant tissues, including leukocytes. For this reason the use of double immunomarcation is essential for the identification of malignant prostate cells. A malignant prostate cell being defined as one which expresses both PSA and P504S. CPCs detected in patients with prostate cancer have been shown to express PSA and P504S (Murray, 2008).

### 3.2 Current methods to detect CPCs: Flow cytometry, CellSearch®, mRNA-RT-PCR, traditional immunocytochemistry

It is beyond the scope of this chapter to review in detail the different methods of detection, two published papers by Paneleaukou et al (2009), and Fehm et al (2005) have extensively reviewed the pros and cons of the different detection methods. In summary, PCR methods have a high rate of false positive results, density gradient centrifugation may be associated with increased lost of circulating cells whereas immunomagnetic separation may not recognize tumor cells which do not express EpCAM and does not differentiate between malignant and benign prostate cells.

In this article we analyze a cohort of patients who participated in a study of prostate cancer detection, comparing the use of serum PSA with the detection of circulating prostate cells and the results of the prostate biopsy (used as the gold standard). The objective was to determine the diagnostic yield of using CPC detection as a sequential screening test in men with a serum PSA and/or DRE considered abnormal.

## 4. Methods and patients

This was a prospectively designed cohort study carried out in the Hospital de Carabineros de Chile (HOSCAR) and the Hospital de la Dirección de Previsión de Carabineros de Chile (DIPRECA), the immunocytochemistry was performed at the Instituto de Bio-Oncology, Santiago, Chile during the period January 2008 and December 2010. The study protocol and written consent form was approved by the ethical committees of all three centers and all patients signed a written consent form. The study was directed with complete conformity to the principals of the Declaration of Helsinki (together with the modifications of Tokyo, Venice and Hong Kong).

All men older than 40 years and attended at HOSCAR or DIPRECA, without a previous history of prostate cancer and fulfilled the criteria's for prostate cancer screening or a prostate biopsy were invited to participate. Biopsy criteria were; serum PSA ≥4.0ng/ml and /or digital rectal examination (DRE) abnormal. Exclusion criteria were older than 85 years and a life expectancy of less than 5 years.

There were 2 groups of men; firstly men attending outpatients, where routine prostate cancer screening was carried out, in addition to the normal PSA test, the men were offered CPC diagnostic testing. These men had no previous history of prostate cancer, and fulfilled

NCCN criteria for screening (2010). The presence or absence of CPCs was compared with the serum PSA level and age.

The second group was formed of men with a suspicion of prostate cancer based on an elevated serum PSA and/or abnormal digital rectal examination, and the blood sample taken immediately before the prostate biopsy. The presence or absence of CPCs was to be compared with the biopsy results, the Gleason score, percent of sample infiltrated with cancer, and number of positive cores. The sensitivity, specificity, positive and negative predictive values were to be calculated. In men with a false negative test the details of the cancer detected would be evaluated.

This second group was analyzed in terms of cost-benefit of using CPC diagnostic testing. In the present analysis the main outcome measure was the incremental cost-utility ratio of using the detection of CPCs as opposed to serum PSA and/or abnormal digital rectal examination to indicate the need for a prostate biopsy, which calculated the saving or additional cost of implementing a screening program based on CPC. The analysis included direct medical costs of the biopsy, direct costs of adverse events (calculated from data obtained from a study conducted in the same hospital), an estimation of indirect costs in terms of lost income, were calculated as days of work lost/average Chilean wage per day as a percentage of the patient group in active employment.

Costs of pre-biopsy tests, biopsy costs (including biopsy kit, ultrasound time, procedure cost, pathology cost, drug cost, hospital bed cost) were obtained from the Hospital Costs Unit Hospital de Carabineros de Chile and Hospital DIPRECA and based on Public Health Service (PHS) list prices in the case of Public Health Patients (FONASA) and Private Health Insurance (PHI) list prices in the case of Private Patients (Isapres). Costs for CPC detection were obtained from the Instituto de Bio-Oncology Costs Unit.

Costs for complications of the biopsy were based on local estimates derived from the Hospital Statistical Unit (Vallejos, 2003). Patients with fever, defined as >38°C were hospitalized and treated with ceftriaxone 1gm iv c/12 for 7 days and metronidazol 500mg c/8 PO for 7 days, hemorrhage was treated with tranexamic acid 500mg c/8 PO for 7 days as an outpatient. Complication rates were 2.9% infection and 0.5% severe hemorrhage. The total cost of the adverse effects was estimated by multiplying the number of biopsies by the frequency of adverse events.

In men with a false positive test for PSA, an estimation of increased follow-up costs was made, this comprised of blood tests for PSA and free PSA and evaluation by the urologist every 4 months, and an estimated 8% of these patients underwent a second biopsy within one year of the first biopsy. In men with a false positive CPC detection, the hospital protocol is repetition of the CPC test with the PSA and free PSA at 4 months and evaluation by the urologist, if the PSA value increased <1.0ng/ml and remained CPC positive a second biopsy was performed. 5 patients had a repeat biopsy.

### 4.1 Sample preparation

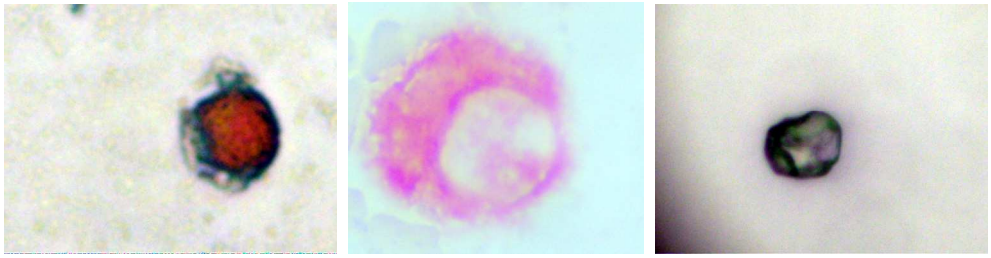
After written informed consent a 4ml blood sample was collected into EDTA (Beckinson-Vacutainer®). The sample was layered onto 2ml Histopaque 1.077® (Sigma-Aldrich) at room temperature, and the mononuclear cells obtained according to manufacturer´s instructions and finally washed 3 times in phosphate buffered saline pH 7.4 (PBS). The pellet was resuspended in 100µl of autologous plasma and 25µl used to prepare each slide (sialinzed DAKO, USA). The slides were air dried for 24 hours and finally fixed in a solution of 70% ethanol, 5% formaldehyde and 25% PBS for 5 minutes and then washed 3 times with PBS.

### 4.2 Immunocytochemistry

Monoclonal antibodies directed against PSA clone 28A4 (Novacastro, UK) in a concentration of 2,5µg/ml were used to detect prostate cells, and identified using a detection system based on alkaline phosphatase-antialkaline phosphatase (LSAB2 DAKO, USA) with new-fuschin as the chromogen. To permit the rapid identification of positive cells there was no counter staining with Mayer´s hematoxilin. Levisamole (DAKO, USA) was used as an inhibitor of endogenous alkaline phosphatase, with positive and negative controls. Positive samples underwent a second stage of processing, using the monoclonal antibody against P504S clone13H4 (Novocastro, UK) and a system of detection based on peroxidase (LSAB2, DAKO, USA) with Vector VIP (Vector, USA) as the chromogen. Endogenous peroxidase was inhibited (DAKO, USA).

### 4.3 Definition of positive samples

A CPC was defined according to the criteria of ISHAGE (Borgen, 1999) and the expression of P504S according to the Consensus of the American Association of Pathologists (Rubin, 2002). A malignant CPC was defined as a cell that expressed PSA and P504S, a benign cell could express PSA but not P504S and leucocytes could be P504S positive or negative but did not express PSA (Figure 1).



**Malignant CPC** **Benign CPC** **Leukocyte**

Fig. 1. Photomicrographs of the different cells type defined by immunocytochemistry.

### 4.4 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 inter-quartile (IQ) 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 Chi-squared for the differences in frequency. For the comparison of variables between more than 2 groups the Kruksal-Wallis test was used. The diagnostic accuracy for the test detecting CPCs was analyzed using standard parameters. For this purpose patients were classified as having or not having prostate cancer. For the purpose of the use of the number of CPCs detected/ml as a diagnostic tool, and only as a mathematical exercise the number of CPCs detected/ml was considered as a continuous variable. A type I error was considered at 0.05, a type II error as 0.20 and the analysis was performed using the Stata 11.0 program.

## 5. Results

**Group 1:** 533 men with an average age of 65.1 ±9.6 years participated in the study, the relation with the detection of CPCs with the serum PSA level is shown in Table 1. There was a significant difference in the frequency of CPC detection in relation to the serum PSA level, Chi-squared for trends p<0.0001, with an odds ratio of 1.00, 2.88.5.02 and 25.60 respectively for the four groups. Comparing individually the four groups there were significant differences, except for comparing men with a serum PSA of 2.0-<3.0 with the 3.0-<4.0ng/ml group.

| | Serum PSA (ng/ml) | | | | |
|---|---|---|---|---|---|
| | <2.0ng/ml | 2.0-<3.0ng/ml | 3.0-<4.0ng/ml | >4.0ng/ml | Total |
| N° Patients | 335 | 101 | 63 | 33 | 533 |
| N° Patients CPC positive | 15 (4.5%) | 12 (11.9%) | 12 (19.1%) | 18 (55%) | 57 (10.7%) p<0.0001 Chi squared for trends. |
| Odds ratio | 1.00 | 2.88 | 5.02 | 25.60 | |

Table 1. The frequency of CPC detection according to serum PSA level

The results of comparing the frequency of CPC detection with age are shown in Table 2. There were no significant differences in the detection of CPCs between the different age groups.

**Group 2:** 228 men participated and underwent prostate biopsy with a mean age of 66.8±8.8 years and a median serum PSA of 5.15ng/ml (IQ 3.2)(Table 3). Of the 228 biopsies, 65 (28.6%) had adenocarcinoma of the prostate detected . CPCs were detected in 71 (31.4%) of all patients, considering men with a prostate biopsy positive for cancer, 86.15% had CPCs detected (Table 4).

| | < 50 years | 50-59 years | 60-69 years | 70-79 years | ≥80 years | Total |
|---|---|---|---|---|---|---|
| N° Patients | 28 | 131 | 214 | 124 | 36 | 533 |
| N° Patients CPC positive | 3 (10,7%) | 14 (10.7%) | 21 (9.8%) | 14 (11.2%) | 5 (13.9%) | 57 (10.7%) |
| Odds ratio | 1.00 | 0.72 | 0.65 | 0.76 | 0.83 | p=0.98 Chi squared for trends |

Table 2. The frequency of CPC detection according to age.

### 5.1 Association between the detection of CPCs and clinical parameters

There was no association between the detection of CPCs and age (p=0.61), but there was an association between the presence or absence of CPCs with the median PSA level (Table 4).

### 5.2 Association between the presence of CPCs and the detection of prostate cancer

86.2% of the patients with prostate cancer on biopsy had CPCs detected. In global terms and statistically significant, patients with cancer and CPCs detected had a higher serum PSA, a higher Gleason score and more advanced clinical stage than those with CPC negative cancer (Table 5).

| | |
|---|---|
| N° Patients | 228 |
| Mean age (years) (SD) | 66.8( 8.8) |
| Diagnosis: | |
| • Cancer, % (n) | 28.6 (65 ) |
| • No cancer ,% (n) | 71.4(163) |
| PSA ng/dl, median (IQR) | 5.15 (3.2) |
| PSA > 4 ng/ml, % (n) | 80.26 (183) |
| CPC presentes, %(n) | 31 (71) |
| • CPC/ml, median (IQR) | 3(3) |
| Cancer stage, % (n) | In 63 patients. |
| • Stage I | 26.98(17) |
| • Stage II | 49.21 (31 ) |
| • Stage III | 20.63(13 ) |
| • Stage IV | 3.17 (2) |
| Gleason, median (IQR) | 5 (2) |

Table 3. General characteristics of the patients. SD: Standard deviation. IQR: Interquartile range. PSA: prostate specific antigen. CPC: Circulating prostate cells. CPC/ml: Circulating prostate cells /ml.

### 5.3 Diagnostic yield of CPC detection

The detection of mCPCs in this cohort correctly identified 86.2% of patients with cancer (95% CI 75.3-93.5), with a specificity of 90.8% (95% CI 85.3-94.8) (Table 6) and an exactitude of 88%. The use of a serum PSA ≥4.0ng/ml and mCPC detection did not significantly improve the discrimination between patients with or without cancer; in fact it reduced the sensitivity from 86.2 to 78.5% (CI 95% 66.5-87.7). The LR+ was 9.36 and LR- was 0.15.Using the number of mCPC detected/ml, instead of a positive-negative score, and a cutoff point of 4cells/ml only increased the specificity by 8%.

### 5.4 Predictive values

(Table 4) The PPV in the complete group of patients (cancer prevalence of 28.5%) was 78.9% (CI 95% 67.6-87.7) and the NPV was 94.3% (CI 95% 89.4-97.3). In the group with a serum PSA <4.0ng/ml (cancer prevalence 13.3%) the most striking result was that of the NPV of 97.1% (CI 95% 84.7-99.9), the rest of the values of predictive estimates were of low precision (Table 6).

### 5.5 Patients false positive

Fifteen men had a false positive result, with a mean age of 63.3±SD7.4 years and a median serum PSA of 4.36 ng/ml (IQR 2.74ng/ml). Two patients had a principal diagnosis of chronic prostatitis and 13 patients benign hyperplasia. Men with a true positive had a

significantly higher frequency of a PSA >4.0ng/ml and higher number of CPCs/ml than false positive men. (Table 5)

| | mCPC positive | CPC negative | p |
|---|---|---|---|
| Patients % (n) | 31.14 (71) | 68.86 (157) | |
| Mean age (SD) | 66.5 (9.5) | 67.0 (8.5) | 0.6955 * |
| PSA ng/ml, median (IQR) | 5.62 (4.64) | 4.93 (3.08) | 0.0402 ** |
| PSA > 4ng/ml, % (n) | 84.51 (60) | 78.34 (123) | 0.279 *** |
| Biopsy<br>(i) no cancer % (n) | 9.15 (13) | 90.85 (129) | 0.0001** |
| (ii) cancer | 86.15 (56) | 13.85 (9) | 0.0001** |
| Gleason, median (IQR) | 6 (2) | 4 (0) | 0.0001** |

Table 4. Comparison of patients CPC positive and negative.

### 5.6 Patients false negative

Nine patients had a prostate biopsy positive for adenocarcinoma in the absence of CPC (Table 7), there were no significant differences between men FN and VN. Comparing men true positive with those false negative, men false negative had significantly lower Gleason scores, earlier stage disease and a discretely lower serum PSA (Table 5).

| | CPCm (+) N=71 | | | CPCm (-) N=157 | | |
|---|---|---|---|---|---|---|
| | Cancer (TP) | No cancer (FP) | p | Cancer (FN) | No cancer (TN) | p |
| % patients (n) | 79 (56) | 21 (15) | 0.0000* | 6 (9) | 94 (148) | 0.0000* |
| Mean age (SD) | 67.1 (10.0) | 64.3 (7.4) | 0.2435** | 68.9 (8.9) | 66.9 (8.5) | 0.4899** |
| PSA ng/ml median (IQR) | 5.96 (4.20) | 4.36 (2.74) | 0.0567*** | 4.80 (0.73) | 4.9 (3.15) | 0.5945*** |
| PSA>4.0ng/ml | 91.1 (51) | 60 (9) | 0.003* | 88.9 (8) | 77.7 (115) | 0.6257* |
| CmCPC median (IQR) | 3.5 (3) | 2 (2) | 0.0000*** | N/A | N/A | |

TP=true positive FP=false positive FN=false negative TN=true negative IQR=interquertile range, N/A=not applicable *Chi squared **T-Test ***Mann Whitney

Table 5. Comparsion between patients mCPC positive and negative.

Total sample Serum PSA <4.0ng/ml

| | Estimation punctual | CI 95% | Estimation punctual | CI 95% |
|---|---|---|---|---|
| Prevalence cancer | 28.50% | 22.7-34.8 | 13.30% | 5.1-26.8 |
| Sensibility | 86.2% | 75.3-93.5 | 83.30% | 35.9-99.6 |
| Specificity | 90.80% | 85.3-94.8 | 84.60% | 69.5-94.1 |
| PPV | 78.9% | 67.6-87.7 | 45.5 | 16.7-76.6 |
| NPV | 94.3% | 89.4-97.3 | 97.1% | 84.7-99.9 |
| LR + | 9.36 | 5.72-15.31 | 5.42 | 2.39-12.28 |
| LR - | 0.15 | 0.08-0.28 | 0.20 | 0.03-1.18 |

NPV negative predictive value LR+ positive likelihood ratio LR- negative likelihood ratio.

Table 6. Diagnostic yield of mCPCs. CI confidence interval, PPV positive predictive value

| Patient N° | Gleason | N° positive cores | % core positive |
|---|---|---|---|
| 55 | 4 (2+2) | 1/12 | 4% |
| 397 | 4 (2+2) | 1/12 | 8% |
| 421 | 3 (2+1) | 2/12 | 5%, 3% |
| 448 | 3 (2+1) | 1/12 | 3% |
| 495 | 3 (2+1) | 1/12 | 3% |
| 498 | 4 (2+2) | 2/12 | 2%, 1% |
| 499 | 5 (2+3) | 1/12 | 5% |
| 715 | 3 (2+1) | 1/12 | <1% |
| 717 | 4 (2+2) | 1/12 | <1% |

Table 7. Details of Patients with prostate cancer and CPC negative.

### 5.7 Patients CPC positive and prostate biopsy positive for cancer

The Gleason scores and clinical stages of the 63 men diagnosed with cancer and who were CPC positive are shown in Table 3.

## 6. Cost-benefit

**Costs:** The summary of the costs are shown in Table 8.

### 6.1 Prostate biopsy

#### 6.1.1 Pre-biopsy blood tests

All patients underwent standard routine blood tests pre-biopsy, with a cost of €37 PHS and €57 PHI.

#### 6.1.2 Drug cost
All patients had prophylaxis with ciprofloxacin 500mg c/12 and metronidazol 500mg c/8 orally for 7 days and a Fleet® enema the morning of the biopsy. Sub-total cost:€15

#### 6.1.3 Prostate biopsy kit
All patients had to bring the biopsy kit, purchased at their own cost €62.

#### 6.1.4 Eco-guided 12 sample prostate biopsy
Costs include ultrasound, biopsy procedure, and pathological evaluation using standard H&E technique, for a cost of €64 PHS and €102 PHI.

#### 6.1.5 Hospital room cost
PHS €16 PHI €122

### 6.2 CPC cost
There is no codification in PHS or PHI costs, we took the price of an immunocytochemical analysis of one tissue as the reference price, PHS €27 and PHI €43.

| | PHS | PHI |
|---|---|---|
| Pre-biopsy blood tests | €37 | €57 |
| Drug cost | €15 | €15 |
| Biopsy Kit | €62 | €62 |
| Prostate biopsy | €64 | €102 |
| Inpatient 1 day | €16 | €122 |
| | | |
| CPC cost | €27 | €43 |

Table 8. Costs of a prostate biopsy: PHS = public health service PHI=private health insurance

### 6.3 Complication cost
Costs were based on the frequency of complications requiring treatment.(table 9)

#### 6.3.1 Sepsis
Estimated cost 228 x 2.9%= 6.61 cases. 7 days hospitalized, PHS €112 PHI €855, antibiotics 7 days €232 Total: PHS €343 PHI €1,087

#### 6.3.2 Hemorrhage
Estimated cost, 228 x 0,5% =1.14 cases Cost outpatient: tranexemic acid 500mg c/8 for 7 days €46

#### 6.3.3 Indirect patient costs (working days)
The average daily Chilean wage is €16, travel costs were not estimated.

### 6.4 Costs for total study population: 228 biopsies

The total cost of the study population of 228 patients with suspicion of prostate cancer either for DRE findings and/or PSA ≥4.0ng/ml is shown in Table 9, the estimated complication costs, include indirect costs. The total cost was divided by the 228 patients to achieve a weighted cost/biopsy.

### 6.5 Costs for study group using CPC detection and omitting biopsies in CPC negative patients

The total cost of 228 CPC detection tests was €6,074 (PHS) and €9,719 (PHI), with the additional cost of 71 biopsies to be carried out in CPC positive men, the total cost for each group is shown in Table 10.

| | **PHS outpatient** | **PHS inpatient** | **PHI outpatient** | **PHI inpatient** |
|---|---|---|---|---|
| Pre-biopsy tests | €8,393 | €8,393 | €12,990 | €12,990 |
| Drug cost | €3,371 | €3,371 | €3,371 | €3,371 |
| Biopsy Kit | €14,179 | €14,179 | €14,179 | €14,179 |
| Biopsy | €14,533 | €14,533 | €23,327 | €23,327 |
| Inpatient<br>Indirect costs | €0<br>€3,660 | €3,633<br>€7,320 | €0<br>€3,660 | €27,860<br>€7,320 |
| Complication costs<br>Sepsis:(N=7)<br>Hospitalization<br>Antibiotics<br>Indirect costs<br>Hemorrhage (N=1)<br>Drug cost<br>Medical control<br>Indirect costs | <br><br>€781<br>€1,621<br>€784<br><br>€45<br>€9<br>€112 | <br><br>€781<br>€1,621<br>€784<br><br>€45<br>€9<br>€112 | <br><br>€5,987<br>€1,621<br>€784<br><br>€45<br>€17<br>€112 | <br><br>€5,987<br>€1,621<br>€784<br><br>€45<br>€17<br>€112 |
| Total<br>228 patients:<br>Per biopsy | <br>**€47,535**<br>**€209** | <br>**€54,828**<br>**€241** | <br>**€66,093**<br>**€290** | <br>**€97,613**<br>**€428** |

Table 9. Cost total of 228 patients and per biopsy according to PHS, PHI in or outpatient.

### 6.6 Costs for study group using CPC detection and omitting biopsies in CPC negative patients

The total cost of 228 CPC detection tests was €6,074 (PHS) and €9,719 (PHI), with the additional cost of 71 biopsies to be carried out in CPC positive men, the total cost for each group is shown in Table 10.

### 6.7 Saving using CPC system

Table 10 shows the total cost for the normal system versus the CPC detection system and savings generated.

| | Normal System | CPC System | Saving |
|---|---|---|---|
| PHS outpatient | €47,566 | €20,877 | €26,689 |
| PHS inpatient | €54,828 | €23,148 | €31,680 |
| PHI outpatient | €66,093 | €30,300 | €35,793 |
| PHI inpatient | €97,613 | €40,115 | €57,498 |

Table 10. Total of normal system versus CPC based system and saving in 228 biopsies.

### 6.8 Costs of false positive tests (in the year after prostate biopsy)

Standard follow up procedure in men with an elevated PSA and biopsy negative for cancer, is a four monthly medical control with serum PSA and free serum PSA and medical control. Control procedure using CPC detection was three monthly medical control, serum PSA and CPC test. The indications for a biopsy within one year were; increase in serum PSA >1ng/ml, number of CPCs/ml increasing.

i. Standard control: serum PSA con percent free PSA: three four monthly blood tests with 3 urology consultations PHS €108 PHI €143. The number of patients in control was 163 men. The number of repeat biopsies, 8%, was estimated from patient activity records of the hospital, the number of estimated repeat biopsy was 13.
ii. CPC detection: serum PSA, CPC detection and urology consultation cost of three four monthly controls PHS €141 PHI €227. The number of patients in control was 15 and there 5 repeat biopsies.

Total cost of follow-up controls: assuming an indirect cost of half a day of work, €8/visit, for a total annual of €24.

i. Standard protocol for 163 men: PHS €21,567, PHI €40,938
ii. CPC protocol for 15 men: PHS €2,480, PHI €3,768

## 7. Conclusions

### 7.1 Patient population

The number of negative biopsies for cancer 71.49%, is similar to that reported in 2 recent studies (Schroder, 2009; Andiole, 2009). The predictive positive and negative values obtained for a serum PSA less and more than 4.0 ng/ml; and the presence of prostate cancer are similar to those previously published. In men with a DRE abnormal and serum PSA <4.0ng/ml 13.3% (6/45) had a biopsy positive for cancer of those men with a serum PSA ≥4.0ng/ml, 32.2% (59/183) had cancer detected (Misky, 2003). We conclude that our patient sample typically represents that of the general screening population.

### 7.2 Diagnostic yield

It is important to emphasize that the detection of CPCs was a sequential test, used in men with a high serum PSA and/or abnormal DRE, therefore a direct comparison with performance diagnosis the serum PSA is not possible. However, an earlier study (Murray, 2010) did not demonstrate a cut-off point for the detection of CPCs in relation to the serum PSA, which is important as it is estimated that approximately 42% of men with prostate cancer have a serum PSA <4.0ng/ml (Lodding, 1995) . Thus the test could be useful to identify men with a PSA <4.0 ng/ml at risk for prostate cancer.

### 7.2.1 Negative predictive value

Probably more important, is that the NPV of 94.3% in a sample of patients with a prevalence of cancer of 28,5% and suspicion of cancer that requires a biopsy, showed that the absence of mCPCs had a high discriminating power. This suggests that men with an increased serum PSA and/or abnormal DRE but mCPC negative could be considered of being at low risk and thus a biopsy might not be necessary. From the point of view of the -LR of 0.15, this permits the reduction of the probability of PC in almost 40% (McGee, 2002) which when applied to a prevalence of approximately 50% significantly reduces the probability of cancer post-test to around 10%. This is clinically useful when determining whether or not to continue investigating a patient. Including, if the cancer was initially missed using the mCPCs test (13.8% of cancers in the study), all the missed cancers were low grade (Gleason 3 or 4, except 1 patient with a Gleason 5 tumor. This patient underwent surgery, the surgery specimen showed a Gleason 5 tumor, infiltrating 5% of 1 lobe, without peri-neural, lymphatic, vascular or capsular invasion, the type of cancer which fulfills NCCN criteria for active surveillance (2010).

## 7.3 Comparison with other methods of CPC detection

The FN result obtained in this study compares with the 24.7% of mCPC negative prostate cancer reported in patients prior to radical prostatectomy and was associated with small low grade tumors and little risk of the presence of bone marrow micrometastasis (Murray, 2010a). This study used the same methodology, defining mCPCs as being P504S and PSA positive.

However, other studies of detection of circulating prostate cells, using a different methodology have been discordant results. Using a dual PSA/prostate specific membrane antigen RT-PCR method Eschwege et al (2009) only found 37% of pre-operative patients to be CPC positive. Davis et al (2008) found no association between CPC detection using the CellSearch® system and the clinical parameters prior to radical prostatectomy or between men with local PC or controls. Likewise in studies using RT-PCR with mRNA PSA no differences were found between patients with localized cancer and healthy subjects in the frequency of CPCm detection (Patel, 2004). We believe that part of this difference is the relatively high detection in control patients. One explication is that CPC can be found in men with prostatitis, however these CPCs are P504S negative (Murray, 2010). This underlies the problem with different methods used to detect circulating tumor cells.

The test using CPCs was designed with a result considered as positive or negative, the incorporation of the number of cells detected/ml increased the specificity by 8% but significantly reduced the sensibility. The CellSearch® system uses a cutoff value of 5 cells/7.5ml of blood to classify a test as positive in patients with metastasis (Davis, 2008; Resel, 2010). However, we consider that in the different stages of a cancer the information needed to make clinical decisions varies. In patients with metastatic cancer the question is one of prognosis, where a determined cutoff value could divide patients in good and bad prognosis, or the change of circulating cell numbers as a measure of response to treatment. In our study the fundamental question was “is there cancer?” Consequently we considered that the presence of single cell is sufficient to classify patients as positive or negative for cancer. Using a cutoff value of 5cells/ml the specificity was 98.77% but the sensitivity decreased to 29.3%, with the utility of the test being significantly decreased.

### 7.4 Application of the test to clinical practice

A prostate biopsy is not without risks to the patient, Rietbergen et al (1997), in a study of 5,802 patients undergoing transrectal prostate biopsy reported an incidence of complications of 0.5% hospitalizations, 2.1% rectal hemorrhage, 2.3% fever and 7.2% persistent hematuria. A study of 381 patients biopsied in the Hospital DIPRECA revealed that 1.57% of patients were hospitalized with fever, treatment was with 7 days of intravenous antibiotics (Vallegas, 2003).
There is an urgent need for an additional diagnostic test which could reduce costs and avoid the risks of unnecessary PB in patients at low risk of cancer; these patients could be actively followed. A persistent increase in serum PSA or the appearance of mCPCs during follow up could be an indication for a biopsy; however, this is yet to be substantiated.

### 7.5 Principal limitations of the study

1. The test was analyzed by one trained cytologist, and as such requires validation with different observers. However, this could be overcome with training and the results could be reproducible between different centers. Equally, the DRE and decision to carry out a biopsy is dependent on the urologist.
2. The study was designed as a sequential test, mCPC detection being requested after the serum PSA and/or DRE, forming a diagnostic test in series. Inspite of this the NPV increased, instead of decreasing as is usual in these types of studies. Although it is unknown the diagnostic yield when comparing with the serum PSA independently and blinded, for which caution is urged before considering the test for routine use, especially for screening, follow up of FN cases or as an isolated tool.
3. The study did not separately analyze the contribution of the serum PSA and/or DRE in the pre-test determination of detecting PC, for which it is unknown the contribution of each in the decision to perform a biopsy. However, this constitutes the daily practice of prostate cancer screening, for which it could be viewed as a strongpoint in demonstrating the diagnostic yield of mCPC detection in the real world.
4. The absence of follow-up of FP patients. Fifteen men had a false positive result for mCPCs, as yet the follow up data with serial serum PSA and mCPCs or a second biopsy are not available. This point is being evaluated in a follow-up study which is currently in progress.

## 8. Cost-analysis

There is consensus in that evidence surrounding new technologies should include cost-effectiveness information. These economic evaluations are part of the daily practice in many countries, such as the United Kingdom. In the case of Latinamerica, including Chile, Pichon-Riviere et al (2008) have shown that there is limited use of the information collected from the evaluations of health technologies, limited resources designated for their development and little government support for these initiatives. In spite of this, countries such as Brazil, Mexico, Chile and Argentina have an active policy of evaluating health technologies and it appears that this is the tendency in other countries in the region (Banta, 2009).
In the process of prioritization and selection of health interventions, included in different packets (public health, community health programs of low and intermediate complexity, special health programs and those of high complexity), the disease frequency and evaluations of cost-benefit play a fundamental role (González-Pier, 2006). Chile has a mixed public-private health system, in that the public health insurance FONASA is financed on the

basis of the social security and fiscal support which covers 70% of the population and a private health insurance system, the ISAPRES which covers a further 16% of the Chilean population (Health Ministry, Chile, 2009).

In this context, our study makes a contribution of the decision making process of incorporating new health technologies. The Chilean male population aged between 45 and 75 years, according the 2003 Census, is estimated to be in 2010 and 2015 approximately 2,296,000 and 2,618,300. Using the results of the First Health Survey of the Health Ministry in 2003, it estimates there will be 95,425 men in 2010 and 116,241 men in 2015 with a serum PSA >4,0ng/ml. However, there is no national record of the number of prostate biopsies performed on an annual basis. The number of patients diagnosed in the public health service between 2005 and 2010 with prostate cancer was 17,719, assuming a positive biopsy rate of 27%, this corresponds to approximately 14,100 biopsies/year in the public health service. This represents 14.8% of the potential population of men with a serum PSA >4.0ng/ml.

Our pilot study has shown that it is possible to eliminate 70% of first time prostate biopsies with the use of the CPC system, which translates into a saving of between €23,874 and €51,807 in the 228 patients who were studied. If the results are confirmed in a larger number of patients this would represent a saving of between €1,465,829 and €3,180,998 per year, assuming an average of 14,000 biopsies/year.

We used a simple standard manual method of CPC detection, in the market there is the FDA approved CellSearch® system for detecting CPCs. However, the costs of the test on the open market are between U$770 and US1,000. We consider that with an experienced immunocytologist the manual method and based on our results the method is acceptable. This means that the cost of installing the CPC program in terms of equipment is of zero cost, as all elements are found in a routine laboratory. The cost per test is much less, €23,50 per test, including labor costs.

Consistent with the findings of others documenting relatively high false-positive rates (Glick, 1998; Sonneberg, 2002; Mohadevia, 2003), we found a substantial number (163/228 ) of those undergoing cancer screening to incur at least one false-positive result, in terms of a serum PSA >4.0ng/ml. The CPC detection test had a significantly lower false positive rate (15/71). The majority of individuals who incurred a false positive screen result received some type of follow-up care in the year following their screening. Despite some individuals not receiving any follow-up care, rates of medical utilization for specific follow-up tests were almost always higher in the false-positive group. This translated into significantly more medical care costs. We calculated that men with a serum PSA >4.0ng/ml and negative first prostate biopsy incurred an average cost of PHS $90,414 and PHI $145,350. The number of men with a false positive CPC detection test is much lower, and although the cost per patient was higher, the overall cost for the system was much less, in terms of costs and medical time. We estimated the number of repeat biopsies taken in these patients from previous hospital data, which further increases costs. When false-positive findings and their consequences are explicitly considered in economic evaluations, model results are often sensitive to the assumed rate of false positive screens (Etiziona, 1995; Chirikas 2002). These results have led some to argue that the cost-effectiveness of different screening programs are primarily driven by rates of false-positive screens among other undesirable outcomes (e.g., over-diagnosis). The reality is that false-positive findings among those undergoing cancer screenings are relatively common, usually constituting the large majority of all positive findings and often leading to follow-up investigations that do not result in a cancer diagnosis (Etzioni, 1995). Given the potential economic and other implications of a false-

positive cancer screen result, it is important that when patients are offered cancer screening it is within a context that allows informed decision-making.

However, despite the convincing evidence in our pilot study of 228 patients, the implementation of CPC detection might result in unanticipated losses or dis-econcomies in the short run. There are two prime reasons, firstly that the new cost-effective technology will probably co-exist with the inefficient alternative for a considerable time period. In our study the idea is a complementary process, leading to decreased biopsies, thus there is not an alternative test; only that CPC detection is not performed. Secondly there might be dis-economies of learning, during the implementation phase, old and new practices may co-exist, with most health professionals being less familiar with new technologies than with the old process. Economies of learning refer to decreasing average cost or increasing average effectiveness, as a result of accumulating experience and know-how. The transition from old to new processes may well cause the opposite effect; increasing average costs or decreasing effectiveness as experience is lacking. Thus patients may have CPC detection performed and regardless of the result proceed to prostate biopsy. The investment necessary to embed the technology in the health organization was not calculated, this would mean capacitating health professionals, information to the patient of the incorporation of new test. That this study was performed as part of a clinical trial, thus had an experimental design, the reality in the clinical situation may be different, and a focus on common practice to order to consider the impact of potentially cost-effective technology on the production processes and budgetary constraints in the health organization.

In summary, we consider that the CPC detection test has an important impact in terms of cost-benefit in the context of a prostate cancer screening program, decreasing the number of deserve to be confirmed with a larger number of patients in an environment of common screening practice.

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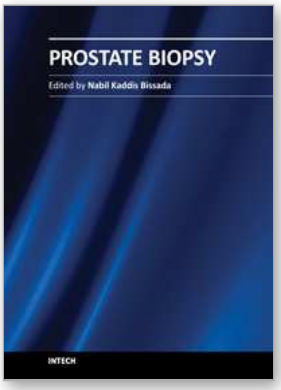


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Prostate Biopsy represents the standard procedure for diagnosing Prostate Cancer. This procedure can be performed transrectally, through perineum or occasionally through the urethra. Although the procedures of Prostate Biopsy are covered in numerous publications, there is still a need for gathering different aspects and methods in one source. Hopefully, this book will help physicians in their effort to provide the best treatment for their patients.



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