

RESEARCH ARTICLE

Efficacy of Using Sequential Primary Circulating Prostate Cell Detection for Initial Prostate Biopsy in Men Suspected of Prostate Cancer

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Abstract

Background: Sequential use of circulating prostate cell (CPC) detection has been reported to potentially decrease the number of unnecessary prostate biopsies in men suspected of prostate cancer. In order to determine the real world effectiveness of the test, we present a prospective study of men referred to two hospitals from primary care physicians, one using CPC detection to determine the necessity of prostate biopsy the other not doing so. **Materials and Methods:** Men with a suspicion of prostate cancer because of elevated PSA >4.0ng/ml or abnormal DRE were referred to Hospitals A or B. In Hospital A all underwent 12 core TRUS biopsy, in Hospital B only men CPC (+), with mononuclear cells obtained by differential gel centrifugation identified using double immunomarking with anti-PSA and anti-P504S, were recommended to undergo TRUS biopsy. Biopsies were classified as cancer or no-cancer. Diagnostic yields were calculated, including the number of possible biopsies that could be avoided and the number of clinically significant cancers that would be missed. **Results:** Totals of 649 men attended Hospital A, and 552 men attended Hospital B; there were no significant differences in age or serum PSA levels. In Hospital A, 228 (35.1%) men had prostate cancer detected, CPC detection had a sensitivity of 80.7%, a specificity of 88.6%, and a negative predictive value of 89.5%. Some 39/44 men CPC negative with a positive biopsy had low grade small volume tumors. In Hospital B, 316 (57.2%) underwent biopsy. There were no significant differences between populations in terms of CPC and biopsy results. The reduction in the number of biopsies was 40%. **Conclusions:** The use of sequential CPC testing in the real world gives a clear decision structure for patient management and can reduce the number of biopsies considerably.

Keywords: Circulating prostate cells - biopsy - prostate cancer - real world testing - comparative effectiveness

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Introduction

Double blind randomised controlled trials are considered to be the most robust form of clinical evidence, aiming to establish efficacy, an unequivocal cause and effect relationship for a very limited number of predefined outcomes in a well defined patient group. However healthcare professionals often do not follow guidelines or recommendations to the letter, daily clinical practice is a complex interplay between experience, training, judgement and draws on data not only from classical randomized trials but also from pragmatically designed studies that may better reflect real life clinical practice. This is especially true with the use of new diagnostic tests. There are dis-economies of learning, those health professionals less familiar with new tests may combine old and new practices; the transition from old to new technologies may decrease effectiveness of a clinical test

until experience is gained.

Sequential use of primary circulating prostate cell (CPC) detection in men with suspicion of prostate cancer has been reported to potentially decrease the number of unnecessary prostate biopsies (Murray et al., 2014), is superior in its predictive value to age related PSA values, PSA kinetics and the Montreal nomogram (Murray et al., 2014a; Murray et al., 2015; Murray et al., 2015a).

We present the findings of a group of men referred from a primary health care system with suspicion of prostate cancer based on an elevated total serum PSA (4.0ng/ml) to one of two hospital urology services. In Hospital A, all men with an elevated PSA and or abnormal digital rectal examination (DRE) detected by the consultant urologist were referred for prostate biopsy. All these men had samples taken for CPC detection immediately prior to biopsy. In Hospital B, prior to biopsy referral, all men with suspicion of prostate cancer underwent CPC testing, as a

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sequential test, the recommendation being that CPC negative men did not need a biopsy and were to be actively followed up, whereas CPC positive men should be biopsied. The ultimate decision being that of the treating urologist as to biopsy the patient irrespective of or based on CPC testing.

Materials and Methods

We prospectively studied all men referred to one of two urology services, Hospital A and B, from primary care services on the basis of an abnormal total serum PSA defined as >4.0 ng/ml.

Hospital A

Men were referred for a TRUS biopsy based on 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, 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.

a) TRUS biopsy: 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 deciated uropathologist.

b) 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 4°C 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 μ L aliquot of autologous plasma. 25 μ L 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.

ii) Immunocytochemistry: CPCs 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 CPC 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 CPC 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 camera 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 CPC count but not if the test was positive or negative. As the test is designed as a positive/negative test it was considered appropriate to proceed, acknowledging that there is an interobserver variation in the absolute mCPC count.

Hospital B

Prior to biopsy, all men underwent CPC testing as described previously, men CPC negative were recommended not to undergo biopsy but to remain under observation with repeated 6 monthly testing of serum PSA and CPC detection testing. Men CPC positive were recommended to undergo prostate biopsy. The final decision as to proceed to biopsy was the final result of the urologist-patient consultation. The TRUS biopsy, biopsy results and CPC determination were as for Hospital A.

Analysis of the results

The discrimination of the diagnostic test in Hospital A 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 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, and whether the CPC test was positive or negative, in order to determine the clinical significance of the CPC test.

In hospital B, the number of actual biopsies avoided was registered and compared with the potential number

Efficacy of Sequential Primary Circulating Prostate Cell Detection for Initial Prostate Biopsy with Suspicion of Prostate Cancer of biopsies that could be avoided in patients attended in Hospital A.

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 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 was 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 study was approved by the hospital ethics committee

Results

During the study period 649 men were referred to Hospital A and 552 to hospital B; the two populations were similar in age 65.5±9.0 years versus 64.9±10.5 years ($p=0.21$) respectively, the median serum PSA 5.50ng/ml (IQR 4.48-7.67ng/ml) versus 5.24ng/ml (IQR 4.26-7.30ng/ml), although a statistically significant difference ($p=0.049$) it was not considered to be a clinically significant difference, and percentage free PSA 15% (IQR 11-22%) versus 15% (IQR 11-22) ($p=0.99$).

a) Hospital A

Of the 649 men undergoing biopsy, 228 men had a biopsy positive for cancer, prevalence of 35.1% (95% CI 31.5-39.0%). The results of CPC testing versus the results of the prostate biopsy are shown in Table 1.

The predictive values of the CPC test in patients

Table 1. Comparison of CPC testing with initial prostate biopsy in Hospital A

	Biopsy positive	Biopsy negative	Total
CPC positive	184	48	232
CPC negative	44	373	417
Total	228	421	649

Table 2. Predictive values of the CPC test in 649 Patients in Hospital A

	Value	95% CI
Sensitivity	80.7%	74.9-85.5%
Specificity	88.6%	85.1-91.4%
Positive predictive value	79.3%	73.4-84.2%
Negative predictive value	89.5%	86.0-92.1%
Likelihood ratio (+)	7.07	5.88-9.31
Likelihood ratio (-)	0.22	0.17-0.28

attended in Hospital A are shown in Table 2. The area under the curve was 0.82 (95% CI 0.79-0.85).

Of the 44 men CPC negative but with a biopsy positive for cancer, 39/44 complied with the Epstein criteria for active observation, twenty nine were Gleason score 4, ten Gleason score 5, two Gleason score 6 and three Gleason score 7 (3 + 4). Of men CPC positive and a biopsy positive for cancer 16/184 (8.7%) complied with the Epstein criteria for active observation.

b) Hospital B

Of the 552 men, 316 (57.2%) underwent prostate biopsy, of whom 194(61.4%, 95% CI 55.8-66.8) had prostate cancer detected for a prevalence in those biopsied of 61.4%, 95% CI 55.8-66.8. The results of CPC testing versus the results of the prostate biopsy are shown in Table 3. Of the six men CPC negative biopsy positive for cancer 4/6 complied with the Epstein criteria, two had a Gleason score of 4, one Gleason score 5, one Gleason score 6 and two Gleason score 7 (3 + 4).

The predictive values of the CPC test in biopsied patients attended in Hospital A are shown in Table 4.

c) Comparing the results between Hospital A and Hospital B

Comparing patients CPC positive and biopsy results (Table 5): There was no significant difference in the frequency of cancer detection on biopsy in men CPC positive ($p=0.09$, OR 0.67 (95% CI 0.41-1.10) RR 0.93 (95% CI 0.86-1.02)). There was no significant difference in the frequency of cancer detection on biopsy in men CPC negative ($p=0.23$, OR 1.75 (95% CI 0.72-4.25) RR 1.67 (95% CI 0.73-3.81)). Nor was there a significant difference between men positive for cancer but CPC negative, 39/44 versus 4/6 ($p=0.19$ Fisher exact test =R 0.26 (95% CI

Table 3. Comparison of CPC Testing with Initial Prostate Biopsy in Hospital B

	Biopsy Positive	Biopsy Negative	Total
CPC positive	188	33	221
CPC negative	6	89	95
total	194	122	316

Table 4. Predictive Values of the CPC Test in 316 Biopsied Patients in Hospital B

	Value	95% CI
Sensitivity	96.9%	93.0-98.7%
Specificity	73.1%	64.1-80.6%
Positive predictive value	85.4%	79.9-89.7%
Negative predictive value	93.6%	86.0-97.4%
Likelihood ratio (+)	3.60	2.68-4.85
Likelihood ratio (-)	0.04	0.02-0.09

Table 5. Comparing the Results between Hospital A and Hospital B

		Biopsy positive	Biopsy negative
CPC (+)	Hospital A	184	48
	Hospital B	188	33
			$p=0.09$
CPC (-)	Hospital A	44	373
	Hospital B	6	89
			$p=0.23$

d) Biopsies avoided

Based on the results of the patients from Hospital A, an ideal biomarker would only detect clinically significant cancers.

The number of non-significant cancers (those which comply with the Epstein criteria), were; CPC (+) Biopsy (+) 16/184 (8.7%); patients CPC (-) Biopsy (+) 39/44 (88.6%) patients; the number of patients with a biopsy negative for cancer was 421. Therefore with an ideal test a total of $16 + 39 + 421 = 476/649$ (73.3%) of biopsies would be avoided. The number of biopsies avoided if only men positive for CPCs were biopsied would be the 417 men CPC negative; accepting that of the 173 clinically significant cancers 5 would not be detected (CPC negative), representing $5/173$ (2.9%) of all significant cancers; and that $48/232$ (20.7%) of those biopsied would not have cancer. In this scenario $417/649$ (64.3%) of biopsies would be avoided.

In the real world situation, represented by Hospital B, the use of sequential CPC detection reduced the number of biopsies by $236/552$ (42.8%), or in real terms that 58.4% of all potentially avoidable biopsies using an ideal biomarker were actually avoided in the real clinical world of urology.

Discussion

Doctors, patients and health care policy makers are faced with medical decisions on a daily basis that often falls short of providing the necessary evidence about comparative harms and benefits of diagnostic interventions (IMNA, 2007). This variation in medical practice which leads to unsustainable healthcare spending with limited association to improved outcomes (Wennberg et al, 1973). The generation and synthesis of evidence that compares the benefits and harms of alternative methods to diagnosis a clinical condition is defined as comparative effectiveness research (CER). It can play a key role in elucidating the relative effectiveness of competing approaches to obtain a diagnosis (Sullivan et al., 2009).

Based on the high negative predictive value of CPC testing and that it did not detect small volumen low grade tumors; the Urology Service incorporated the test in its guide for prostate cancer screening. The limitations in the sensitivity and specificity of total serum PSA values remain problematic (Welch et al., 2005). In men with a total serum PSA of 4.0-10.0ng/ml, 70% will have a biopsy negative for cancer, assuming the inherent risks of a prostate biopsy (Rietbergen et al, 1997).

The test was designed to give a positive or negative result, with a clear decision structure for patient management and a clear position of the test in the management pathway. Thus for effective patient management the CPC test has a high clinical utility in that it produces actionable results to ameliorate adverse outcomes caused by prostate biopsy, criteria suggested by Groose et al for diagnostic biomarkers (Grosse et al., 2006). The diagnostic benefit of the test is its position in the management pathway, in that patients have undergone PSA testing and DRE evaluation by a urologist, both low

cost tests with minimal side effects. The next step is the prostate biopsy, the first invasive diagnostic test. The 90% negative predictive value for the CPC test is for predicting when there is not a clinically significant prostate cancer in men with suspicion of a cancer. Its use in all men would not be appropriate or clinically useful; men with low PSA values or normal DRE may never require consideration for a prostate biopsy. This underscores the need for a well established clinical guide including clinical decision making based on the test's results.

The results from Hospital A are similar to those reported previously (Murray et al., 2014), what is important is that in the same screening population, the results of CPC testing versus biopsy result were not significantly different, as shown in Table 5. Initial concerns were not about false positive tests, which are approximately 20% and usually as associated with acute prostatitis. The use of double immunomarcacion reduces this error, other methods based on EpCAM have failed to detect a difference with control patients and early localized prostate cancer, possibly for this reason (Eschwege et al., 2009; Davis et al., 2008). The concern was about false negative tests and that clinically significant prostate cancer could be missed. The results of Hospital A are similar to those reported previously (Murray et al., 2014), in that 2% of all significant cancers are missed. This has to be put in context that, 15% of all prostate cancers occur in men with a PSA of less than 4.0ng/ml (Fang et al., 2001; Horninger et al., 2004), and 20% of men with an initially negative biopsy have prostate cancer detected at the second biopsy (Ploussard et al., 2013). As men with a negative biopsy continued to be followed up in the Urology Service and not in Primary Care, this worry has decreased.

The diagnostic benefit of CPC testing is also a function of its degree of complementarity; further additional tests to recommend prostate biopsy are not required to reach a clinical decision. Complementarity is closely related to the tests specificity.

The existence of diagnostics based upon test results is not sufficient, the test must be accesible and acceptable; physicians and patients must use the test; make decisions based upon the test, and have access to the appropriate intervention (Grosse et al., 2006). Previous work (Murray et al., 2014; Murray et al., 2015) and the results from Hospital A provided sufficient evidence to produce clear guidelines for the use of the CPC test. The access to test results are timely and do not produce a significant delay in the management program.

Other new biomarkers that have been proposed for the diagnosis of prostate cancer, include the detection of cell free circulating DNA (cfDNA). The use of cfDNA as a biomarker for prostate cancer has been reported, with conflicting results. Although levels of cfDNA were significantly elevated in metastatic prostate cancer, there was no difference found between benign disease and localized cancer (Jung et al., 2004). While Boddy et al. (2005) reported that levels were higher in benign disease as compared to that in cancer patients. However, using the levels of the non-cancer gene prostaglandin-endoperoxidase synthetase 2, those patients with cancer had significantly higher levels detected (Ellinger et

Efficacy of Sequential Primary Circulating Prostate Cell Detection for Initial Prostate Biopsy with Suspicion of Prostate Cancer al., 2008), similarly others have found significantly higher levels of cfDNA in cancer patients (Bastian et al., 2007; Gordian et al., 2010), especially when using hypermethylation specific PCR (Altimari et al., 2008). As with CPCs, a critical factor for determining standard cfDNA cutoff values is to establish uniform methods for sample collection, processing, DNA extraction, cfDNA quantitation and analysis, as well as marker gene (Xue et al., 2009). That the use of fragmentation patterns, which differ in patients with cancer as compared to those with benign disease, allelic imbalances, methylation patterns and the use of tumor specific DNA sequences may improve its role in the diagnosis of prostate cancer (Ellinger J et al., 2011). Furthermore it has been reported that the presence of cfDNA is associated with the presence of CPCs and could be used as a marker for CPCs (Schwarzenbach et al., 2009).

One relative draw-back of cf-DNA methodologies is the high implementation costs and need for a highly specialized work force, not normally available in a district hospital. However, comparative effectiveness research does not have to include costs, although many comparative effectiveness research studies utilize economic measures such as costs, it must be noted that care must be taken to distinguish between the two (Weinstein et al., 2010). A cost-benefit study of CPC detection showed that the cost of the test was more than offset by a reduced number of biopsies and the hospitalizations for adverse effects (Murray et al., 2013). The reduction of 42% in the number of prostate biopsies is significant, in terms of direct costs and waiting times for invasive procedures. The test is low cost and can be implemented in the routine immunocytochemical laboratory of a district hospital. One limitation of our study is that it is a single centre, in that all samples are processed in a centralized laboratory, however this decreases the tests variability in that adequately trained personal, manage, process and inform the tests.

Conclusions: The use of primary CPC detection in a real world situation decreases the number of prostate biopsies by approximately 40%, it's positive/negative result gives a clear decision structure for patient management and a clear position of the test in the management pathway. Further large scale multicentre studies need to confirm these results.

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